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Nasal Eosinophilia in So-called Bacterial Hypersensitivity in Asthmatic Children

Preliminary Report

by KJELL AAS

The question of bacterial hypersensitivity in bronchial asthma has occasioned much controversy. Some allergists claim that bacteria or parts of the bacteria act as genuine antigens. Others believe that infections act solely as provocative factors. The former school of thought advocates the use of bacterial vaccines while the latter is more negative in approach to such treatment. This controversy is partly a result of our inadequate methods for proving or disproving the existence and/or significance of bacterial hypersensitivity.

In the Children's Department of the Oslo University Hospital some investigations related to these problems are being carried out. This is a preliminary report of some of the results.

A central question raised is whether any patients with bronchial asthma show specific allergic reactions to bacterial vaccines, i.e. do the bacterial vaccines in use contain any significant antigen.

Theoretically an increase of the eosinophilia in the nasal secretion after application of a test extract may signify that the extract carries antigenic qualities unless it is strongly irritative to the mucosa. This seems true in specific allergies. Corresponding reactions could be expected in possible bacterial hypersensitivities causing bronchial asthma at least in the proportion of cases in which the mucosa of the nasal cavity is representative of the bronchial mucosa as regards eosinophilia.

In acute exacerbations of bronchial asthma an increase in nasal eosinophilia is commonly found. The exacerbation may be regarded as a positive reaction to unwanted and unplanned provocations having much in common with planned ones. Parallels may be drawn.

After numerous examinations for nasal eosinophilia in connection with provocation tests in specific hypersensitivities, we have started a search for corresponding reactions toward the bacterial vaccines commonly used.

Material

For practical purposes we started to examine specially selected patients. This was necessary principally because the patients had to be closely observed in the Hospital for some time. For the examinations, those patients have been picked who presumably

suffered from "bacterial allergy" satisfying the following conditions: (1) recurrent attacks of bronchial asthma following upper respiratory tract infections such as common colds etc., (2) tendency to attacks or exacerbations of the allergic symptoms within the first few days of such infections, (3) no traceable specific hypersensitivities that may be connected with the patients symptoms (after thorough examinations including skin testing and provocation procedures)

Of 153 asthmatic children seen in the Out Patient Department or hospitalized during a 8 months period, 29 patients were selected as satisfying the criteria mentioned Sixteen children were under 4 years of age and fourteen of these were considered unfit for the studies. These omitted patients however were examined with respect to their reactions to vaccine injections. Fifteen patients f ll filled all conditions stated and were tested with the methods described (Group A)

Parallel to these the same methods were used in testing 13 other asthmatic children with known specific hypersensitivi ties (Group B)

An additional group of 16 randomly ected asthmatic children was tested with single applications. These latter patients suffered from partly known specific hyper sensitivities and in most of the cases the asthmatic attacks were precipitated by common colds (Group C)

Methods

From the older children the secretion was easily obtained by allowing the patient to blow the nose in a soft plastic "handker chief" and partly by gently swabbing the nasal mucosa with a thin cotton tipped metal string. When the swab was used, it was firmly pressed against the mucosa of the posterior nasal cavity for a minute or so. The latter method was uniformly employed in the youngest age groups.

The secretion was spread on a slide with as few manipulations as possible, dried in air without heating and stained with the May Grünewald stain for 3–5 minutes. To obtain optimum staining buffered water with a pH 5.8 was used as a solute. The slide was examined under the immersion oil lens and with optimal light conditions. For analysis the examiner must rely on a method of grading the amount of eosinophilia. A scale modified after Hansel, Andersen & Koch was found valuable (1 7)

Nasal secretion was obtained several times during the first two days of the in vestigational period. From each swabbing at least two slides were made. The eosinophilia was judged, and the slide with the highest grade picked as control for the later ones.

Local application One nostril was tamponed, gently but firmly and the tampon was repeatedly saturated with the fluid in question. The fluids employed in this series were: *Controls* (1) 0.9 % sodium chloride, (2) Coca solution, (3) pollen or house dust extracts, (4) "vaccine" made of indifferent diphteroids; *Test series* (5) anticatarrh vaccine SIFF (stock vaccine) (6) staphylococcus aureus vaccine Bencard, (7) autogenous vac cine containing staphylococcus aureus or vaccine of isolated staphylococcus aureus from the patient in question. For the nasal applications all vaccines were employed undiluted and the fluids applied in the sequence mentioned

The tampon was removed *after a few* hours (usually 3–5) and was as a rule found to be covered with mucus which was used for examination. Secretion was at the same time obtained by the methods described, and this was repeated at intervals during the following 48 hours. A new tamponade was then done unless a persisting increase in the eosinophilia had been found. In the latter case no new tamponade was done until repeated checks showed an eosinophilia t the starting value or lower

Systemic application. Finally the patient received a subcutaneous injection of fluid no 4 with subsequent examinations of the nasal secretion during 4 days, and on the 5th day an injection of fluid nos. 5, 6 or 7 was given followed by nasal swabbings. The dose given in injection was variable. Those pa

tients who showed no eosinophilic response on local applications were given 0.5 ml of the 1/1 or 1/10 dilution. Those with presumed positive reactions were given 0.5 ml of dilutions of 1/10000. To save hospitalization time the completing tests had to be performed while ambulatory in a few cases. None of these belonged to the positively reacting group.

Results

Of the 15 patients in Group A, 3 had to be omitted because of persistent high grade eosinophilia during the entire hospital stay. Two other children showed increased eosinophilia on the control extracts and not on the test extracts. These reactions were found after application of house dust extract and Coca solution respectively, partly giving a clue to specific hypersensitivities not formerly found in these patients. Seven of the remaining patients showed no eosinophil response whatsoever.

Three patients, however, reacted with a significant eosinophilic increase after application of the test fluids and not on the controls. The positive responses were reproducible. In two of the three patients a marked rise in the secretion eosinophilia was also found after the injection of autogenous vaccine and stock vaccine respectively. The maximum rise here was found after 36 and 48 hours. Focal reactions including sibilous sounds on auscultation were encountered in the same two patients.

In the third patient no change in eosinophilia was found after the injection and no focal asthmatic reaction was observed following it. This patient, however, acquired an otitis media of the "catarrhal" type on the tested side 24 hours after the local application of the stock vaccine.

It is noteworthy that the patient with the most marked eosinophilia (and focal reactions) had received stock vaccine injections regularly during the preceding three years with several instances of moderate to severe asthmatic reactions following even minute doses.

In Group B none of the 13 children reacted to the bacterial vaccines. Among the 5 patients suffering from known hypersensitivity to house dust, four showed increased nasal eosinophilia after the house dust application. One of these patients suffered from slight asthmatic symptoms including scattered sibilous sounds on auscultation the day after the bacterial vaccine injection (dose 0.5 ml of the 1/10 dilution). A slight temperature rise and some tenderness at the injection site was observed in several instances following the bacterial vaccine in this group as well as in the other groups. A similar reaction was found after fluid number 4 as well. In no case in Group B was an eosinophilic response found after the injections.

The Group C patients were tested with intranasal vaccine applications only. Control applications were not done. Fifteen patients showed no eosinophilic response. One child, however, clearly reacted positively on the first stock vaccine application. When retested no change in eosinophilia could be found on repeated examinations. The eosinophilia first encountered may have been caused by accidental circumstances such as chance dust exposure or a slight infection.

The 14 children under 4 years given stock vaccine injections reacted with

some tenderness locally and/or a slight temperature rise None of them reacted with exacerbations of the allergic disease

Several of the children in all groups had formerly received stock vaccine injections, some undiluted and others in minute doses. *No significant differences* between these and the unvaccinated ones could be found except in the one positive child in Group A.

Discussion

The etiologic diagnosis of bacterial allergy is for the present time not well based More objective criteria should be sought for Bergquist has experimented with combined extracts from mucus membranes and bacteria (2) The experiments are encumbered with some weak links however and ascertained results are still awaited The leukocyte incubation method of Blatt (3) is applicable in the determination of bacterial cellular antigenicity the delayed reaction type In bronchial asthma the tissue response is of the atopic immediate reaction type and no conclusions can be drawn from the cellular antigenic qualities of bacteria (4)

Our method has weak links as well. On the one hand, technical difficulties are met with. Eosinophilia in secretions is very often difficult to judge quantitatively Counting in percentages is not satisfactory and a rough estimate must frequently be relied on The increase in eosinophilia noted in our study has been so convincing however that the examiner is left without doubt On the other hand, some uncertain eosinophilic changes have occurred in the 'negative group Such uncertain changes have not resulted in any sort of hypotheses here although some of them might have some significance

Rajka (8) claims that the nasal mucosa as an allergic responding organ is not representative for the mucosa behind the nasal cavity including the bronchial tree. *Certainly it is true that increased nasal* eosinophilia does not necessarily indicate an allergic state in the bronchi. We are nevertheless convinced that in children the allergic state of the bronchi most frequently is associated with an allergic reactivity of similar quality in the nasal mucosa. This opinion is obviously shared by many allergists, and Rysing has recently shown that clinically observable allergic manifestations in the nose and bronchi combined are found in 67 % of children on provocative testing (9) In bronchial hypersensitivities of a specific type the nasal mucosa most frequently reacts allergically to the antigen in question A study in this Hospital of the frequency of nasal eosinophilia in asthmatic children is in progress. One hundred and eighteen patients are hitherto included in this series. Of the children suffering from severe asthma every one shows nasal eosinophilia. Seventy-eight % of those with moderate asthma and about 60 % of those with mild asthma show eosinophilia in the nasal secretion. Fourty-eight patients were examined in this respect when having symptoms and all but four showed pathological nasal eosinophilia.

More questionable is whether the positive reactions described in connection with the vaccine applications indicate that the patient suffers from a true bacterial hypersensitivity In the nasal cavity bacteria are normally found in astronomic numbers, and if one accept the hypothesis of signi-

ficant bacterial antigenicity these bacteria theoretically may be responsible for the pathological secretion with eosinophilia found even between asthmatic attacks. We find difficulties in explaining *how* the application of proportionately inconsider able amounts of bacteria may release an allergic reaction. The fact that the reaction is reproducable with the bacterial vaccine while not with the controls suggests nevertheless that the vaccine employed contains an antigen of significance Changes forced on the bacteria through the vaccine preparation process as for example crushing decomposition or release of incorporated antigenic substances may theoretically play a role. Non-specific protein components must also be taken into consideration.

A specific allergic reaction toward the bacterial vaccines could easily be explained in patients who previously had received similar vaccine injections. In such cases an assertion that the vaccinations contrary to the purpose had sensitized the patient, could rest uncontradicted. Examples of such appropriate sensitizing effects are known to most allergists (5) One of our patients lends himself to such argumentation, but the other two do not

Non-specific irritations (thermal, mechanical and chemical) may cause an increase in the *secretion eosinophilia* as well as clinical exacerbations of the allergic disease The positive reactions described may be due to irritative properties of the test solution. When such reactions are not observed in the control experiments *as for example after the application* of diphteroid vaccine it is tempting to assume that the "irritation" is synonymous with "antigenic quality

This assumption is supported by the findings of eosinophilic increases after the parenteral injections of the vaccine that showed activity on nasal applications, compared to the failure to respond to control fluid injections. Support is also found in the observation of asthmatic symptoms provoked by test vaccine injections in minute doses.

The observations obtained stimulate further investigations in this important field.

Summary

A preliminary report is given of some studies concerning bacterial vaccines in asthmatic children. A method is introduced in which nasal eosinophilia is studied in connection with local applications of bacterial vaccines and controls. A reproducable positive response is found in three patients of a selected group suffering from presumed bacterial allergy" In two of these patients focal asthmatic reactions are observed following the injection *of minute doses of the* vaccine in question. The method is briefly discussed.

References

1 Aas, K Eosinofili i sekret. *T Norske Lægeforen 77* 1237 1958.
Bergquist G A contribution to the problem of autosensitization in bronchial asthma. *Acta Allerg 10* 157 1956.
3 Blatt H The identification of specific allergens in bacterial allergy and their use in desensitization. *Ann Allergy 10* 53, 1952.
4. Feingold B. F Infection in bronchial allergic disease *Pediat Clin N Amer 6* 709 1959

5. Flensborg, S. W.: Om formentlig bakteriell allergisk asthma hos barn og dennes behandling. *Ugeskr Læg 11* 12: 357 1950.
6. Glaser, J.: Allergy in Childhood. C. C. Thomas. Springfield 1956.
7. Koch, H.: Allergical investigations of chronic otitis. *Acta Oto Laryng (Stockh) Suppl. 62* 1947
8. Rajka, F. Allergie und allergische Erkrankungen. Budapest 1959
9. Rybring, E.: The results of provocative tests with inhalant allergens in asthmatic children. *Acta Allerg 14* 423, 1959

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Precipitating Antibodies in Human Sera from Different Age Groups and in Colostrum as Determined by Streptococcal Antigens with Diffusion-in-Gel Methods

by LARS Å. HANSON and STIG E. HOLM

The development of the serum proteins which show antibody activity, also called the immune globulins (13, 14), have recently been studied by immune electrophoresis (24, 25). It has been observed that γ-globulins appear in the serum of the fetus after 12 weeks of pregnancy. The β_{1A} and β_{2M}-globulins which also belong to the immune globulins are not found in the serum of the fetus and newborn but they appear during the first weeks after parturition. The γ-globulins of the newborn are derived from the mother via the placenta as shown by studies with labelled γ-globulins (19, 20). Due to the disappearance of these γ-globulins the level in the serum of the child decreases after birth. At about the age of three months an increase of the γ-globulin level can be observed as the γ-globulin of the child counteracts the elimination (27). The γ-globulin level then slowly increases to the adult value.

This investigation is an attempt to reflect this development of the immune globulins by a study of precipitating antibodies against β-hemolytic streptococci in different age groups—*fetal*, newborn, child and adult.

Material

Antigens. Filtrates of 18 hour cultures of β-hemolytic streptococci (strain S 84, Lancefield group A, Griffith Type 3) grown in beef heart infusion broth have been used. These antigen preparations were concentrated about ten times by lyophylization or by pervaporation before use.

Antisera. An immune serum from a horse immunized with β-hemolytic streptococci (strain Dochez N Y 5, Lancefield group A, Griffith Type 1) was used as a reference serum. Immune sera from sheep immunized with human blood plasma were used to localize the different *protein fractions* of the electro-separated materials in the immune electrophoretic analyses.

Patient material. Umbilical cord sera were obtained from legal and spontaneous abortions after 12–40 weeks of pregnancy as well as at full term deliveries. Sera were taken from the corresponding mothers. The material also included sera from children varying in age from a few days to 15 years. Sera from normal adults were used as a comparative material.

All of the sera tested were taken from individuals without any recent history of streptococcal infections. The antistreptolysin O titer was determined and sera with a titer of 200 Todd units/ml or less were chosen. In some sera, mainly the fetal sera, the titer was not determined.

Sera from individuals with an antistrepto-

lysin O titer of 400 Todd units/ml or more were analyzed for comparative purposes.

Colostrum samples were taken from some of the mothers within one day of parturition.

Methods

The *double diffusion-in-gel* analyses were performed according to Ouchterlony (*8), as slightly modified by Hanson (9). The micro modification developed by Wadsworth (3*) was also used to some extent. The *immune electrophoretic technique* of Grabar & Williams (5, 6) was employed in the modification reported by Wadsworth & Hanson (33). To demonstrate antibodies present in too low quantity to give visible precipitates with the standard immune electrophoretic technique a specially devised *comparative immune electrophoretic technique* described by Wadsworth & Hanson (33) was used. The antistreptolysin O determinations were performed at the Bacteriological Laboratory, Sahlgren Hospital. The streptolysin O preparation used for the determinations was the same as the reference antigen employed in this study. In the routine method of this laboratory the results are recorded as titers 50, 100, *00 etc. Todd units/ml.

Results

In the comparative double diffusion experiments a reference spectrum was used which was formed by the streptococcal antigen and the reference horse immune serum. The spectrum consisted of twelve lines which were numbered I–XII (Fig. 1). Eleven of these were demonstrated to be separate serological systems, while systems IX and X seemed to be serologically related. Some of the antigenic factors represented by these lines have been identified with known streptococcal antigen preparations (16). Thus the lines I–IV were found to be con-

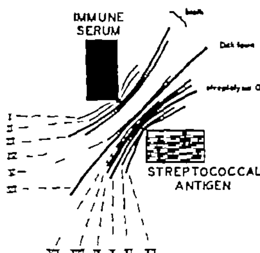


Fig. 1. Diagrammatic representation of the reference spectrum formed by the streptococcal antigen preparation and the reference serum. The precipitation lines are numbered I–XII and the identified systems are indicated.

nected with antigenic factors in the broth used for the cultures. The line VI identified with a purified Dick toxin, the line X with a pure streptolysin O preparation and the line XI with streptococcal DNAse. The reference spectrum did not show any line correlated to the M-antigen. The comparative double diffusion analyses of the sera from fetuses, newborn and mothers with the reference spectrum were performed in five basin plates. As may be seen in Fig. 2 the antigen was placed in the two lower basins, the reference serum in the central upper basin and the two sera from the fetus or newborn and the corresponding mother were placed in the basins on each side of the reference serum basin. The sera from children and adults were compared with the reference spectrum in three basin plates.

Several of the precipitating antibodies in the analyzed sera could be identified by comparison with the reference spec

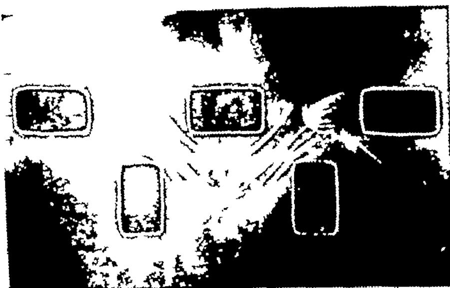


Fig. 2. Phot of comparative double diffusion analysis where the sera from mother (right upper basin) and her child (left upper basin) are compared with the reference serum (central basin) by means of the streptococcal antigen (lower basins).

Table 1 *Serum components demonstrated in blood sera from d fferent age groups by comparative analysis with the reference spectrum*

Age groups	Identified serum components I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Unidentified components	Number of cases
13–17 weeks														9
18–20								4	3	6	4	3	4	16
1–40					2			7	7	8	5	5	8	9
Newborn					1	1	1	12	11	14	8	8	11	15
months					3		1	3	6	5	2	3	10	13
3 8								1	2	4		1		18
1														13
1 5–2 ears								1	2	1	1		2	18
3–5					1			3	5	6	5	1	4	33
6 9							1	8	5	7	2	3	8	11
9 1					1	1	1	9	11	14	8	10	7	18
13 16					1	1	1	9	14	18	12	11	11	19
Adul					9	1	1	26	23	32	13	18	19	33
Total					1	4	6	80	89	112	60	60	83	[illegible]

trum (Table 1) The lines I–IV which corresponded to antigenic factors in the broth did not identify with any lines formed with the human sera Antibodies corresponding to the systems V, VI and VII were only found in a few cases while antibodies corresponding to the systems VIII, XI and XII were found in about a third of the examined sera. The precipitation lines IX and X which may represent only one

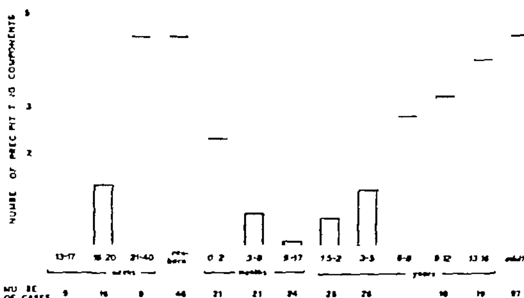


Fig. 3. Graphic representation showing the average number of precipitating antibody components found in 342 human blood sera from different age groups. (This material includes 123 sera which were compared with another antiserum and therefore could not be inserted in Table I.)

serological system were the most frequently identified systems in the analyzed material. Some of the demonstrated precipitation lines could not with certainty be identified by means of the used reference serum. Qualitative differences between the identified antibody components in the various age groups could be noted. Differences between the total number of precipitating antibodies demonstrated in these groups were also observed (Fig. 3). In the fetal material taken after 13–17 weeks of pregnancy no serum contained precipitating antibodies against β hemolytic streptococci but after 18–20 weeks of gestation the fetal sera averaged 1– precipitating antibodies. In the group of fetuses aborted after 21–40 weeks of pregnancy an average number of 4–5 precipitating antibody components was found. The same number was found in the umbilical cord sera from newborn delivered at full term. A decreasing frequency of antibodies was found after birth, and the lowest number was obtained in sera from children 9–17 months of age. After the age of 1.5 years the average number of antibodies gradually increased to the same figure as found in the adult material.

The comparison between the antibodies in the sera from the mothers and the corresponding umbilical cord sera from the fetuses and newborn gave some information. The sera from the mothers of the fetuses aborted after 18–20 weeks all contained a large number of antibodies while in only half of the corresponding fetal sera precipitating antibodies were demonstrated. The average number of antibody components in the fetal sera from this age group was lower than that found in the

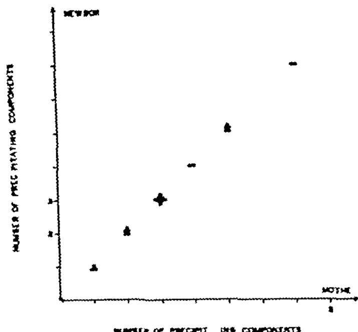


Fig. 4. Diagram showing the correlation between the number of precipitating antibody components found in the blood sera of 34 mothers and their newborn children.

mothers' sera (Fig. 3). The comparison in the next age group of 21–40 weeks of pregnancy indicated that the same number of antibodies was found in the corresponding maternal and fetal sera. This correlation was also demonstrated by the fact that the comparative analyses with the reference spectrum identified mainly the same antibodies in the maternal and fetal sera. A similar comparison between the sera from newborn and their mothers showed a perfect positive correlation regarding the number as well as the identity of the demonstrated lines (Fig. 4). In 15 of these cases antistreptolysin O determinations were performed. No difference could be found between the mothers and their offspring.

The relation between the titer of the single antibody antistreptolysin O and the more complete antibody content as determined by the Ouchterlony technique was studied. An increasing number of precipitating antibodies was found with an increasing antistreptolysin O titer as seen in Fig. 5.

A possible source of immune globulins for the child may be the mother's milk. For this reason colostrum samples were analyzed for the presence of antibodies revealed by our streptococcal antigen with the diffusion-in-gel techniques. In the 42 samples analyzed precipitating antibodies were demonstrated in about 50 %. Only one antibody component was found in most of the colostrum specimens and the same antibody among several others was seen in the blood serum of the mother.

Immune electrophoretic studies were

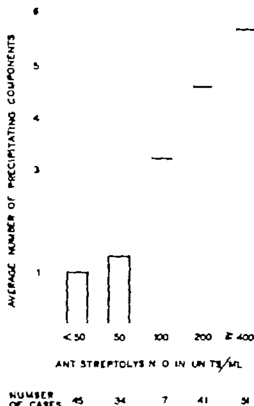


Fig. 5. Graphic representation showing antistreptolysin O titers correlated to number of precipitating antibody components in 218 human sera.

included in the investigation in order to determine the electrophoretic characteristics of the demonstrated immune globulins. Some sera from each age group were examined and altogether 45 sera were analyzed in this respect. The sera were electrophoretically separated and thereafter analyzed with the streptococcal antigen (Fig. 6). The position of the different serum protein fractions after the electrophoresis was determined by an anti-human plasma immune serum. In some instances the comparative immune electrophoretic technique had to be used in order to demonstrate the precipitating serum antibodies. They were found to be localized mainly in the β_2 and in some cases also further out into the γ globulin region. In a few cases the precipitates were localized more toward the β_1-globulin area. No consistent differences could be observed between the precipitates obtained with the sera from the various age groups. Some colostrum samples were also analyzed in this way. As a rule it was not possible to demonstrate the antibodies in

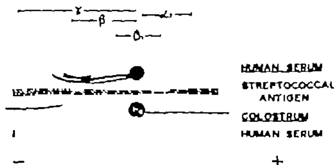


Fig. 6. Schematic representation of an immune electrophoretic analysis of human serum and comparative immune electrophoretic analysis of colostrum showing the localization of streptococcal antibodies. The electro-separated serum and colostrum were analyzed with a streptococcal antigen. The colostrum was supplemented with a serum containing streptococcal antibodies in order to obtain a visible precipitate. The localization of the serum fractions, as indicated in the upper part of the figure, was determined with an anti-human plasma immune serum. (The separated materials are underlined.)

the colostrum with the standard immune electrophoretic technique since they were present in too low quantity to form visible precipitates. Thus the comparative modification of the immune electrophoresis was utilized. Such an analysis is shown in Fig. 6 where electro-separated colostrum was analyzed with the streptococcal antigen diffusing from a longitudinal basin parallel to the direction of the electro-separation. Additional antigenic material containing the same antibody as the colostrum in a higher concentration and diffusing from the other longitudinal basin was required to demonstrate the presence of the antibodies in colostrum. The antibodies against β hemolytic streptococci found in colostrum were mainly localized in the β_2-globulin region.

Discussion

The problems concerning the transfer of antibodies from mother to offspring have been reviewed by Vahlquist (29 30). It is there stated that antibodies are principally transferred to the fetus in utero. Vahlquist Lagercrantz & Nordbring (31) have shown that antistreptolysin O antibodies appeared in the blood of the fetus after the middle of gestation. We found precipitating streptococcal antibodies in fetal sera before 20 weeks of pregnancy as reported in a preliminary note (15) This is in accordance with the findings of γ-globulins in fetal sera after 1 weeks of pregnancy (4 25) The passage of γ-globulins through the placenta from the mother to the fetus is well illustrated by the good correlation we found between the antibodies demonstrated in the sera from mother and fetus as early as after 1 weeks of gestation. This relationship persisted until at birth a perfect positive correlation was obtained when identical antibodies were found in the sera from the mother and newborn. In addition the characteristics observed in immune electrophoretic analyses were *mainly the same in all of the age groups* The antistreptolysin O titers were the same in the analyzed pairs The lastmentioned finding is not in agreement with a report by Stück & Sliomon (26) and others concerning streptolysin O titers in sera from newborn and mothers but seems to be supported by the studies of Adamson Lödgren & Malmnäs (1) The titre steps in our antistreptolysin O determination may have been too great to point up minor differences in levels of antibodies. By the diffusion in-gel techniques it was possible to compare not only a single but several streptococcal antibodies—still no differences were noted. This may be in accord with the fact that the γ-globulin levels of the mother and newborn are the same (21) The only immune globulins that exist in the fetal serum before birth are γ-globulins, while the β_{2A} and β_{2M}-globulins not appear until after parturition (13 25) This means that from an immunological point of view the streptococcal antibodies are likely to be γ-globulins and that they are not related to β_{2A} or β_{2M}-globulins.

After birth the elimination of the passively acquired antibodies was reflected by a decreasing number of precipitating antibodies in the sera from children. The decreasing part of the curve (Fig 3) is presumably related to the half life of γ globulins reported to be about 20 days (3 34) After an average value of compo-

nents which was almost zero in the age group of 9–17 months an increase was observed which illustrates the phase of the active immunization. The level of the adult was then gradually reached. Between the age groups 3–5 and 6–8 years a marked increase was noted (Fig 3) This may reflect repeated streptococcal infections at the beginning of the school age

Antistreptolysin O antibodies in human colostrum have been studied (18 22) In addition by employing diffusion-in gel methods other streptococcal antibodies have here been investigated. They were found to be localized in about the same region as the serum antibodies after immune electrophoresis. The immune globulin fraction of human milk has been shown to contain γ- β_{2M}- and β_{2A}-globulins (7 10 11) The streptococcal antibodies in the milk belonged to the γ-globulins as did the serum antibodies. The antibodies human milk are considered, however to be of negligible importance to the child (20 21 22, 23, 30) although some kinds of antibodies occur in high amount in the milk and do not seem to pass the placenta to any great extent (9 18)

A few investigators have used diffusion in-gel methods to analyze streptococcal antibodies in human sera (4 6 9 12) With sera from normal adults Halbert Swick & Sonn found between 0 and 4 lines, Hanson found between 1 and 7 with an average of 3 to 4 lines. Bonilla Soto & Pomales-Lebrón reported an average of 45 lines for a similar material In a material of sera from individuals with streptococcal infections or rheumatic fever Halbert *et al.* found between 2 and 7 lines, while Hanson found between 2 and 9 Similar results were obtained by Harris, Harris & Ogburn who used the Oudin technique. The present investigation on a larger material confirms the earlier results. The results of the analyses of the sera from children and newborn babies are mainly in agreement with the findings of Bonilla-Soto *et al.* obtained from a similar material. The fact that in this study a higher number of lines were found in the analyses of human sera than in the other reports may be due to differences in the used techniques and antigen preparations Refilling of the basins in the double diffusion analyses has been done. This procedure has been reported to give rise to artefacts in some instances (17) That no such artefacts have influenced the presented results has been controlled by the use of the known reference spectrum included in all performed experiments.

Streptococcal infections during the phase of active immunization give an immune response that is reflected by the demonstrated gel precipitation patterns. The immune response seems to be the result of repeated infections over a long period of time and is therefore not remarkably affected by acute infections as shown by the diffusion in-gel methods (9) This was also indicated by the fact that a lack of streptococcal antibodies was detected only in the sera from the youngest children while sera from adults with few exceptions contained several antibodies. That some changes in the gel precipitation patterns occurred after streptococcal infections was evident however as some correlation between the number of lines and various antistreptolysin O titers existed (Fig 5) The antistreptolysin O antibody was the most frequently identified antibody in the analyzed sera. This suggests

that the antistreptolysin O determination may be a good indicator of the presence of streptococcal infections.

Summary

The development of the immune globulins was studied by means of the determination of precipitating antibodies against β-hemolytic streptococci in sera from different age groups—fetal, newborn, child and adult. The analyses were performed with diffusion-in-gel methods.

In fetal sera after 18–20 weeks of gestation precipitating antibodies were found, but in a lower number than in the sera from the corresponding mothers. The same number of antibodies were found in the maternal and umbilical cord sera after 21–40 weeks of gestation as well as at parturition. The antistreptolysin O titer was also the same in the sera from the mothers and newborn. From birth till the age of 9–17 months a decreasing number of antibodies was seen. From this age a slow increase was noted and the level which was found i the adult material was approached in the age group of 12–16 years. The same immune electrophoretic characteristics was demonstrated for the antibodies in the different groups of sera as in colostrum.

Finally a positive correlation was found between the number of precipitating antibodies and the antistreptolysin O titers.

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References

1 Adamson C A., Lönnqvist B. and Malmros, C Antibodies in mothers and newborn infants. *Scand J Clin Lab Invest* 3 52–57 1951
2. Anson H., Baltensberger, M. and Olitzki, A. L.: The appearance of antibacterial and antitoxic antibodies in maternal sera, umbilical-cord blood and milk. Observations on the specificity of antibacterial antibodies in human sera. *Pediatrics*, 23 86–91 1959
3. Barr, M., Glenny A. T and Randall, K. J Concentration of diphtheria antitoxin in cord blood and rate of loss in babies. *Lancet* 2 324–326, 1949
4 Bonilla-Soto O and Morales-Lebrón A.: Variation with age of antistreptococcal antibodies and their correlation with antistreptolysin O titers in human sera. *Proc Soc Exp Biol Med*, [illegible] 337–340, 1953
5. Grabar, P et Williams, C. A.: Méthode permettant l'étude conjuguée des propriétés électrophorétiques et immunochimiques d'un mélange de protéines. Application au sérum sanguin. *Biochim Biophys Acta* 10 193–194, 1953.
6. — Méthode immuno-électrophorétique d'analyse de mélange de substances antigéniques. *Biochim Biophys Acta* 17 67– 4, 1955.
Gugler, E. und von Muralt G Über immunoelektrophoretische Untersuchungen an Frauenmilchproteinen. 2. Mitteilung. *Schweiz Med Wschr* 89 925–929 1959

8. HALBERT S. P., SWICK L. and SONN C.: The use of precipitin analysis in agar for the study of human streptococcal infections. II. OUCHTERLONY and OAKLEY techniques. *J Exp Med*, *101* 557–576, 1955.
9. HANSON L. Å. Immunological analysis of streptococcal antigen and human sera by means of diffusion-in-gel methods. *Int Arch Allergy* *14* 279–291 1959
10. — Immunological analysis of human milk. *Int Arch Allergy* *15* 245–256, 1959.
11. — The serological relationship between human milk and blood plasma. *Int Arch Allergy* *17* 45–69 1960.
12. HARRIS, T. N., HARRIS, S. and OGBURN C. A.: Gel precipitation of streptococcal culture supernates with sera of patients with rheumatic fever and streptococcal infections. *Proc Soc Exp Biol Med* *90* 39–45, 1955.
13. HITZIG W. H. Die physiologische Entwicklung der Immunglobuline (Gamma und Beta$_2$-Globuline) *Hel Paediat Acta* *12* 596–605, 1957
14. HITZIG W. H. SCHEIDEGGER, J. J., BÜTLER, R., GUGLER, E. und HÄSSIG, A.: Zur quantitativen Bestimmung der Immunglobuline *Helv Med Acta*, *26* 142 161 1959
15. HOLM, S. and HANSON L. Å.: The presence of precipitating antibodies against β-hemolytic streptococci in sera from different age groups—fetal, newborn and child. 12th Scand. Congr of Pathology and Microbiology Gothenburg June 1959 *Acta Path Microbiol Scand* (in press).
16. — To be published.
17. KAMINSKI M.: Quelques observations sur la technique de précipitation spécifique en milieu gélifié d'Ouchterlony I. Influence de la recharge en antigène. *Bull Soc Chim Biol (Par)*, *36* 279–288, 1954.
18. MALMNÄS C. LÖFGREN S. and ADAMSON C. A. Om övergång a[illegible] antikroppar från modern till fostret och det nyfödda barnet. *Svensk Läkartidn*, *48* 1737–1741 1951
19. MARTIN DU PAN R., SCHEIDEGGER, J. J. WENGER, P. KOECHLI, B., ROUX, J. et RABINOWITZ, J. Le passage transplacentaire de la gamma-globuline chez la femme. *Et Neo-natal* *7* 71–80, 1958.
20. MARTIN DU PAN R., SCHEIDEGGER, J. J. WENGER, P. KOECHLI, B. und ROUX J. Das Verhalten der intramuskulär intravenös und per os verabreichten Gammaglobuline *Blut* *5* 104–114, 1959
21. VON MURALT G. und GUGLER, E.: Die Reifung der Immunglobuline. *Helv Med Acta*, *26* 410–423, 1959
22. NORDBRING F. The appearance of antistreptolysin and antistaphylolysin in human colostrum. *Acta Paediat (Upps)*, *46* 481–496 1957
23. — The failure of newborn premature infants t[illegible] absorb antibodies from heterologous colostrum. *Acta Paediat (Upps)*, *46* 569–578, 1957
24. SCHEIDEGGER, J. J. MARTIN E. et RIOTTON G. L'apparition des diverses composantes antigéniques du sérum au cours du développement foetal. *Schweiz Med Wsch* *86* 224–226, 1956.
25. SCHEIDEGGER, J. J. et MARTIN DU PAN R.: Étude immuno-électrophorétique des protéines sériques du nouveau-né et du nourrisson. *Et Néo-natal* *6* 135–144 1957
26. STÜCK, B. und SILOMON R.: Vergleichende Untersuchungen zum Antistreptolysingehalt im Serum von Müttern in der Schwangerschaft und bei der Geburt sowie von ihren Neugeborenen. *Mschr Kinderheilk* *108* 6–1.., 1960.
27. ORLANDINI, O. SASS-KORTSAK, A. and EBBS, J. H.: Serum gamma globulin levels in normal infants. *Pediatrics*, *16* 575–584, 1955.
28. OUCHTERLONY Ö.: Diffusion-in-gel methods for immunological analysis. *Progr Allergy* *Vol. 5* pp 1–78 (KARGER, Basel/New York 1958).
29. VAHLQUIST B.: Placental transfer of antibodies in human beings. *Et Néo-natal*, *1* 31–45, 1952.
30. — The transfer of antibodies from mother t[o] offspring *Advanc Pediat* *Vol. 10*, pp 305–338 (Year Book Publishers, Chicago 1958).
31. VAHLQUIST B., LAGERCRANTZ, R. and NORDBRING, F. Maternal and foetal titres of antistreptolysin and antistaphylolysin at different stages of gestation. *Lancet*, *2* 851–853 1950.
32. WADSWORTH C. A slide microtechnique for the analysis of immune precipitates in gel. *Int Arch Allergy* *10* 355–360 1957
33. WADSWORTH, C. and HANSON L. Å.: Comparative analysis of immune electrophoretic precipitates employing a modified immune electrophoretic technique *Int Arch Allergy* (in press)
34. WIENER, A. S.: The half-life of passively acquired antibody globulin molecules in infants. *J Exp Med*, *94* 213–221 1951

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Congenital Obstructive Emphysema in Infants

by M. EIKEN

Localized pulmonary emphysema is relatively common in infants with respiratory infections, following aspiration of foreign bodies and less common as a result of compression of a bronchus by enlarged lymph nodes or a lung cyst. However a few cases of localized emphysema which appear to be of congenital origin have been reported.

The aetiology is generally believed to be some form of obstruction of a bronchus the passage of air being relatively unhindered during inspiration, but inhibited or completely blocked during expiration. Thus, aspiration of viscous mucus in the course of pneumonia may cause atelectasis or emphysema, according to whether the obstruction is complete or incomplete. In congenital emphysema various forms of obstruction have been demonstrated. The commonest finding is a defective development of the cartilaginous rings in areas of the bronchial wall (4 7 9 15 16 19 20). In other cases the cause was thought to be a redundant mucosal flap acting as a check valve (8 13 18) compression due to a patent ductus arteriosus (8) or a dilated vein (18) or stenosis without demonstrable changes of the cartilaginous tissue (10). In 14 out of the 50 recorded cases of congenital emphysema, however no obstruction was found (1 3 6 8, 11 12, 15 18 20 22, 23, 24, 25) despite the fact that operation or autopsy had been performed in all but 7 of these cases. In 2 instances kinking of a bronchus was believed to be an aetiological factor (12 14). That an abnormal development of the elastic tissue of the lung might be responsible in some of these cases has also been considered (23) but this presumption has never been confirmed.

A few patients have shown signs of heart disease (10 20), two had a large defect in the anterior mediastinum (9 14) and in one case a diaphragmatic hernia was revealed (24). As a rule however this form of congenital emphysema does not appear to be accompanied by other developmental anomalies.

Recently a few cases of a special form of emphysema have been reported, i.e. obstructive emphysema comprising the entire right lung or in exceptional cases both lungs. This kind of emphysema is due to compression of the right main bronchus or of the distal end of the trachea by an anomalous course of the left pulmonary artery (5 17 and others). This anomaly differs from those mentioned

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9. HANSON L. Å.: Immunological analysis of streptococcal antigens and human sera by means of diffusion-in-gel methods. *Int Arch Allergy 14* 279–291, 1959.

10. — Immunological analysis of human milk. *Int Arch Allergy 15* 245–256, 1959

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12. HARRIS, T N, HARRIS S. and OGBURN C. A.: Gel precipitation of streptococcal culture supernates with sera of patients with rheumatic fever and streptococcal infections. *Proc Soc Exp Biol Med 90* 39–45, 1955.

13. HITZIG, W H.: Die physiologische Entwicklung der "Immunglobuline (Gamma und Beta$_2$-Globuline) *Hel Paediat Acta 12* 596–603, 1957

14. HITZIG W H., SCHEIDEGGER, J J BUTLER, R., GUGLER, E. und HÄSSIG A.: Zur quantitativen Bestimmung der Immunglobuline. *Hel Med Acta, 26* 142 151 1959.

15. HOLM, S and HANSON L. Å.: The presence of precipitating antibodies against β hemolytic streptococci in sera from different age groups—fetal, newborn and child 12th Scand. Congr of Pathology and Microbiology Gothenburg June 1959 *Acta Path Microbiol Scand* (in press).

16. — To be published.

17. KAMINSKI, M.: Quelques observations sur la technique de précipitation spécifique en milieu gélifié d'Ouchterlony I. Influence de la recharge en antigène. *Bull Soc Chim Biol* (*Par*) *36* 279–298, 1954.

18. MALMNÄS, C, LÖFGREN S. and ADAMSON C. A.: Om overgång av antikroppar från modern till fostret och det nyfödda barnet. *Svensk Läkartidn, 48* 1737 1741 1951.

19. MARTIN DU PAN R., SCHEIDEGGER, J J WENGER, P KOECHLI, B., ROUX, J et RABINOWITZ, J Le passage transplacentaire de la gamma-globuline chez la femme. *Et Neo natal, 7* 71–80 1958.

20. MARTIN DU PAN R., SCHEIDEGGER, J J WENGER, P KOECHLI, B und ROUX, J Das Verhalten der intramuskulär intravenös und per os verabreichten Gammaglobuline *Biol 5* 104–114, 1959

21. VON MURALT G und GUGLER, E.: Die Reifung der Immunglobuline. *Helv Med Acta, 26* 410–423, 1959

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24. SCHEIDEGGER, J J MARTIN E. et RIOTTON G L apparation des diverses composantes antigéniques du sérum au cours du developpement foetal. *Schweiz Med Wschr 86* 224–226, 1956.

25. SCHEIDEGGER, J J et MARTIN DU PAN, R.: Étude immuno-électrophorétique des protéines sériques du nouveau-né et du nourrisson. *Et Néo-natal, 6* 135–144, 1957

26. STÜCK, B und SALOMON R.: Vergleichende Untersuchungen zum Antistreptolysingehalt im Serum von Muttern in der Schwangerschaft und bei der Geburt sowie von ihren Neugeborenen. *Mschr Kinderheilk, 108* 8-12, 1960

27. ORLANDINI, O SASS-KORTSAK, A. and EBBS, J H.: Serum gamma globulin levels in normal infants. *Pediatrics, 16* 575–584, 1955.

28. OUCHTERLONY Ö Diffusion-in-gel methods for immunological analysis. *Progr Allergy Vol. 5* pp 1–78 (KARGER, Basel/New York 1958).

29. VAHLQUIST B. Placental transfer of antibodies in human beings. *Ét Néo-natal, 1* 31–45 1952.

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31. VAHLQUIST B., LAGERCRANTZ, R. and NORDBRING F Maternal and foetal titres of antistreptolysin and antistaphylolysin at different stages of gestation. *Lancet, 2.* 851–853, 1950.

32. WADSWORTH, C.: A slide microtechnique for the analysis of immune precipitates in gel. *Int Arch Allergy 10* 355–360, 1957

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Recently a few cases of a special form of emphysema have been reported, i.e. obstructive emphysema comprising the entire right lung or in exceptional cases both lungs. This kind of emphysema is due to compression of the right main bronchus or of the distal end of the trachea by an anomalous course of the left pulmonary artery (5 17 and others). This anomaly differs from those mentioned

above in being frequently associated with other vascular malformation such as an abnormal origin of the arteries from the aortic arch dextroposition or coarctation of the aorta, persisting left superior vena cava or septal defect

The clinical signs of congenital localized emphysema generally appear within the first months of life characterized by respiratory difficulty often with prolonged expiration usually a dry cough and not infrequently cyanosis. Often nutritional disturbances are the result of the dyspnoea These signs are due to the emphysema causing a more or less pronounced compression atelectasis of the other parts of the lungs primarily homolaterally but often also contralaterally the heart and mediastinum being displaced towards the good side Infection of the respiratory tract i generally not present initially and if so one may doubt congenital origin On the other hand, secondary infection aggravating the condition is not uncommon As a result the extent of the emphysema may vary but it is characteri tic that without treatment the disease runs a chronic course and that complete pontaneou regression seems to be exceptional.

Probably milder cases of congenital obstructive emphysema may occur without clinical sign in infancy a several reported cases of obstructi e emphysema in adult appear t have been of congenital origin ([illegible] and others)

At the Copenhagen County Hospital Gentofte w hav observed 3 cases of localized emphyse f apparently congenital origin which will be reported here

[illegible] ... 2½ months, [illegible] 193 [illegible]

gation had revealed enlargement of the liver and spleen.

H had been born 3 weeks prematurely but the birth history was otherwise normal. His birth weight was 3400 g and his length 50 cm. Prior to admission he had been in good health except that he failed to thrive

On admission he wa a thin, but otherwise normal looking infant weighing 4100 g with a slight, dry cough, but normal temperature The li er and spleen were palpated as fa as 3 to 4 cm below the costal border but no other abnormal ties were observed. Laboratory examinations, bone marrow exa minations, and repeated d fferential blood counts f iled to reveal any abnormalities. Moro reaction and Wassermann reaction were negative Throughout his st y in hospital, the boy seemed t be entirely unaf fected, but he took his feedings only with reluctance and continued t thrive poorly The first X ray examination of the chest taken * months after admission disclosed a distinctly increased air content in the left hemithora a w ll as a displacement of the heart and mediastinum to the right (Fig 1) Repeated X-ray examination during the remainder of his stay showed these findings almost unchanged. Since the pulmonary changes were interpreted a a possible left sided pneumothorax, puncture f the left pleura in two sites was ttempted bout 2½ months after admission bu t without result

During the subsequent 5½ months the temperature was somewhat fluctuating but apart from this there were no abnormal findings. When the temperature had been normal for one month the patient was discharged H wa then about 1 year old

H was re-ad itted t the age of ears 3 ths, because he st ll failed to thrive His lengt w now 84 c [illegible] weight 10 kg (erage 89 c 12.2 kg) The l er and sple were st ll palpable nd X-ray [illegible] [illegible]

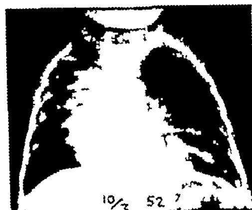


Fig. 1. Case 1. N. B.-C. Chest at 4½ months of age. Severe emphysema of left upper lobe. Over the left diaphragm the shadow of the compressed lower lobe. Displacement of the heart and mediastinum to the right and depression of the diaphragm.



Fig. 2. Case 2. T.H., aged 1 month. Unmistakable emphysema of the left upper lobe with displacement of the great vessels to the right and flattening of both leaves of the diaphragm.

infection, and similar attacks continued during the subsequent 2 months. Bronchography showed basal displacement of the bronchial tree on the left side and filling of only the central 2 cm of the left upper lobe bronchus.

Left-sided thoracotomy disclosed severe emphysema of the upper lobe so lobectomy was performed.

On examination of the specimen the emphysema was found to involve predominantly the anterior and apical-posterior segments whereas the lingula was only moderately affected. A check valve was demonstrable. Microscopic examination revealed apart from the emphysema inflammatory changes in the bronchi and bronchioles. No anomaly of the cartilaginous rings in the bronchial tree was found.

After discharge the boy was as if transformed. He thrived well and was much more lively than previously. When last seen, 4 months after the operation, he was still making good progress.

Case 2. T H. A boy 10 [illegible] old, was transferred in 1958 from [illegible] maternity department because of chondrodystrophy and suspicion of heart disease. His birth was normal except for slight asphyxia, but the following 3 days he had repeated attacks of cyanosis, and at one time he showed signs of cerebral hypoxia. During the subsequent days, he rallied, but was still cyanosed when crying.

On admission, he showed distinct retraction along the costal borders during respiration, but no cyanosis at rest. There was no elevation of temperature and no signs of respiratory infection. Physical examination showed his head to be strikingly large compared with his trunk, and h[illegible] mild micrognathia and palato[illegible] noted. His chest was deformed, the [illegible] being short and very prominent [illegible] mild kyphoscoliosis of the [illegible] Auscultatory changes over the [illegible] lungs were heard. Limbs [illegible] both elbow joints were [illegible] tens on. His hands and feet [illegible] his spleen and liver [illegible] the costal borders. X-[illegible] the chest failed to show [illegible] heart but the great [illegible] the right and there [illegible] left lung (Fig. 2) [illegible] changes were seen [illegible] f 8 days. X-ray [illegible]



Fig 3. Case –, T H aged 5 months. Bronhography showing constriction and incomplet filling of a bronchus t the left upper lobe at its site of origin from the main bronchus. The other bronchial branches to the left lung are displaced basally



Fig. 4 Case 3, K.F., aged 2 days. Pronounced emphysema of the left upper lobe with compression atelectasis of the left lower lobe and right upper lobe. The arrows indicate the outlines of the left lung Marked rotation of the heart and depression of the diaphragm.

d changes corresponding to those seen hypoplastic achondroplasia. S bluxation both lbow joints was observed.

While in hospital, the patient had numerous attacks of cyanosis, especially when crying and taking food. He developed a cough, gradually with production of ample mucus. Repeated aspirations were of merely transient effect and most of the time he had to be kept an oxygen tent. Gradually the condition mproved so that, despit continued respiratory difficulty he could be discharged to his home when a l ttle over 2 months old.

When 4½ months old he was re-admitted because of upper respiratory infection of 8 days duration. His condition was about the same as on his first admission. Bronchography revealed narrowing of the central part of the left pper lobe bronchus with incomplete peripheral filling whereas the remaining branches of the bronchial tree were displaced basally (Fig 3) The examination also roused suspicion of a similar narrowing of a right middle lobe bronchus.

Since repeated X-ray examinations showed increasing emphysema of the left upper lobe, left-sided thoracotomy was performed when the patient was 5½ months of age. The apico-pectoral part of the left upper lobe was found t be highly emphysematous and extended through the anterior mediastinum into the right hemithorax. Resection of the anterior and apical posterior segments was performed. Postoperatively the lung expanded well, but despit intermittent artificial ventilation through a tracheal t be the patient condition deteriorated and he died 6 days later

Examination of the removed segments of lung showed the segmental bronchi t have been out peripheral to the narrowing visualized by bronchography On histological examination the lung tissue was emphysematous, but without pneumonic changes or pecific inflammation. The bronchi and bronchioles were somewhat ectatic

Autopsy revealed bilateral pneumothorax, with total collapse of both lungs, and cor pulmonale



Fig. 5. Case 3, K.F. aged 3 days. On lateral view of the chest the left upper lobe may be seen forcing itself in front of the heart and elevating the sternum. Marked depression of the diaphragm.



Fig. 6. Case 3, K.F. 11 days after resection of the left upper lobe. The drain has just been removed, and some pleural changes still remain at its former site in the apex, but the left lower lobe has expanded satisfactorily.

CASE 3. K.F. A boy 1 day old, admitted in the spring of 1960. After delivery, which was uncomplicated, he had cried spontaneously. His birth weight was 3320 g and his length 51 cm. A few hours later his breathing became laboured, and he developed cyanosis when crying.

On admission, auscultation revealed the respiratory sounds over the left hemithorax to be diminished, the heart displaced to the right, but no murmurs. The liver and spleen were distinctly palpable. E.C.G. indicated right axis deviation, but no other definite abnormalities. X-ray examination of the chest revealed severe emphysema of the left upper lobe, the heart being displaced backward and to the right (Figs. 4 and 5) and the left lung extending through the anterior mediastinum far into the right hemithorax. Moreover there was an unmistakable depression and flattening of the diaphragm.

During the subsequent days, further exacerbation occurred. The infant turned cyanotic upon the slightest exertion and required pure oxygen. Repeated X-ray examination showed increasing emphysema of the left lung, and at the age of 4 days thoracotomy was carried out as an emergency procedure. The emphysematous left upper lobe was removed and the bronchus cut at its site of origin from the main bronchus.

The postoperative course was uneventful. The compressed right upper lobe and left lower lobe expanded well, the cyanosis ceased completely, and 17 days after the operation the patient could be discharged (Fig. 6).

The removed lobe showed a short constriction of the main bronchus to the upper lobe about 4 mm peripheral to where it had been severed. At this site the lumen was less than 1 mm in diameter, while otherwise the bronchi were slightly ectatic. Microscopic examination showed the cartilage to be normally developed. The lung tissue was emphysematous, but without inflammatory changes.

Discussion

According to previous reports congenital emphysema has often given rise to great diagnostic difficulties and many cases have been erroneously diagnosed at first. The *differential diagnosis* includes mainly (1) obstructive emphysema due to other causes, especially in pneumonia and aspiration of foreign bodies (2) compensatory emphysema in pneumonia atelectasis or hypoplasia of other lung segments, (3) pulmonary cysts (4) pneumothorax, (5) diaphragmatic hernia, and (6) heart disease

Most of these conditions are characterized by compromised function of parts of the lung As a result the clinical signs, dyspnoea cough, and varying degrees of cyanosis, as well as the auscultatory findings are uncharacteristic and radiography of decisive diagnostic importance

Congenital emphysema practically always involves the upper lobes or the right middle lobe. The lower lobe does not appear to have been involved except in one case (3) As another characteristic it is nearly always lobar whereas infectious obstructive emphysema generally manifests itself in the form of small localized translucencies or else especially in staphylococcal pneumonia, develops into cyst like cavities, the so-called pneumatoceles These changes may vary in appearance from day to day and as a rule they soon yield to conservative treatment.

Aspirated foreign bodies, if not radio-paque and visible on a plain film may usually be demonstrated by bronchography or bronchoscopy

In cases of cysts, the diagnosis may usually be made by demonstrating the wall of the cyst and here tomography may be helpful Incidentally congenital emphysema may often run a more alarming course than the cysts (3)

Pneumothorax may generally be ruled out by demonstrating the scanty but homogeneous pattern of the emphysematous lung Most difficulties are encountered *in cases of partial pneumothorax* in which case screening and oblique exposures may be required.

Diaphragmatic hernia ought not to give rise to much diagnostic difficulty At times, a contrast medium may have to be given orally in order to confirm the diagnosis.

Differentiation from compensatory emphysema is usually possible by screening the chest. In compensatory emphysema the heart and mediastinum become displaced away from the emphysematous side during inspiration, while in obstructive emphysema they are fixed or shifted toward the emphysema during inspiration. Furthermore in obstructive emphysema there is usually depression and flattening of the diaphragm with reduced excursions.

Extended knowledge of congenital lobar emphysema and of the differential diagnostic problems should in most cases permit the correct diagnosis by comparing the results of repeated X ray examinations with the history and clinical course.

A congenital aetiology in the present cases is indicated first and foremost by the apparent absence of primary pulmonary infection. It is characteristic, moreover that in all three cases the signs appeared immediately after birth in one only in the form of failure to thrive but in the other two as dyspnoea and cyanosis In all three cases the liver and spleen were found to be

palpable below the costal border no doubt because both leaves of the diaphragm were depressed

There is reason to stress the chronic course in the first two cases Obstructive emphysema arising in the course of pneumonia usually disappears rapidly within days or weeks whereas congenital emphysema takes a chronic and not un commonly progressive course in most of those cases which have been observed. A few cases with spontaneous, but in complete remission have been reported (3 11 22)

Secondary respiratory infections are not uncommon in congenital emphysema and the bronchitic changes found in the lobe removed from Case 1 do not militate against a congenital origin

In the other two patients, the assumption of a congenital aetiology is further supported by the absence of inflammatory changes in the removed lobes and by the bronchographic and pathological findings respectively Considering the chondrodystrophy in one of the cases, the possibility of localized chondromalacia of the bronchial tree being responsible for the bronchographic changes is present although it was not demonstrated histologically

T eatment

In order to prevent deformity of the hest secondary infections leading to cut exacerbation and not least the risk of complications due to hypoxia surgery is usually indicated as soon as possibl It should consist in removing the em physematous segment of the lung fter which the infant generally recover and devel p normally Only a few of the pa tients (11 15 *0) appear to have remained particularly prone to respiratory infections leading to transient emphysematous chan ges in the lungs.

In a few cases, attempts have been made to relieve the discomfort by direct puncture of the emphysematous segment of the lung Kornbold & Baker (12) used this procedure successfully in two cases which remained free of recurrence for 6 months In other cases, this treatment has been ineffective (9 11 15 18) and in two it has even given rise to complicating tension pneumothorax with a fatal issue As a result most authors now advise against this method.

Summary

Three cases of congenital emphysema in infants are reported. All had clinical signs from birth and were treated by operation at the age of 2½ years, 5½ months, and 4 days respectively Likely causes of the emphysema in the form of narrowing of the respective bronchi, were found in only two of the patients, one of whom was also suffering from hypoplastic achondroplasia

Aetiology clinical features and dif ferential diagnosis are discussed nd the importance of early diagnosis and treat ment is stressed.

Obstructive emphysema of congenital origin has to be considered as a differential diagnostic possibility when an infant shows signs of respiratory disorder con sisting in dyspnoea cough, and possibly cyanosis during the first months of life

The diagnosis is based primarily upon the radiographic changes in conjunction with the history and clinical course

As a rule the course is chronic. In most cases the treatment is surgical, viz resec tion of the emphysematous segments

References

1 Bierring, A.: Et tilfælde af lobert obstruktionsemfysem hos et spædbarn. Nord Med 18 1375–1376, 1941
2. Brünner, S. A. and Pock-Steen O Chr: Localised emphysema. Acta Radiol 33 184–190 1950.
3. Caffey J.: On the natural regression of pulmonary cyst during early infancy Pediatrics 11 48–64 1953.
4 Castrelli, J F Hipoplasia de un bronquio pedicular y enfisema lobar agudo en un lactante. Hoja Tisiol 4 230– 40 263–276 1944
5. Conrad, S., Miller, R. A., White, H. and Potts, W J Bronchial obstruction due to pulmonary artery anomalies. I Vascular sling. Circulation, 17 418–423 1958
6. De Bord R. A. and Shelley C. E.: Congenital obstructive emphysema treated by lobectomy Amer J Dis Child 85 775–778, 1954
7 Ferguson C F and Neuhauser, E. B. D Congenital absence of the lung (agenesis) and other anomalies of the tracheobronchial tree. Amer J Roentgenol 52 459–471 1944
8. Fischer, H. W Potts, W J and Holinger P H.: Lobar emphysema in infant and children. J Pediat 41 403–410 1952.
9 Gross, R. E. and Lewis, J E.: Defect of the anterior mediastinum. Surg Gynec Obstet 80 549–554, 1945.
10 Hamilton L C. and Gillespie, R. W Congen tal hypertrophic emphysema. Amer J Roentgenol 80 421–4..5, 1958.
11 Holzel, A., Bennett E. and V ughan B. F Congenital lobar emphysema. Arch Dis Ch ldh 31 216–221 1956.
12. Korngold, H. W and Baker, J M.: Non surgical treatment of unilobar obstructive emphysema of the newborn. Pediatrics, 14 296–303, 1954.
13. Leahy L. J and Butsch W L.: Surgical management of respiratory emergencies during the first weeks of life. Arch Surg, 59 466–482, 1949.
14 Lewis, J E. and Potts, W J Obstructive emphysema with defect of the anterior mediastinum. J Thor Surg, 21 438–443, 1951
15. Nelson T Y.: Tension emphysema in Infants. Arch Dis Childh, 32 38–41 1957
16. Overstreet R. M.: Emphysema of a portion of the lung in the early months of lif Amer J Dis Child, 57 861–870, 1939.
17 Potts, W J Holinger, P H. and Rosenblum, A. H.: Anomalous left pulmonary artery causing obstruction to the right main bronchus. JAMA, 155 1409–1411 1954.
18. Robertson R. and James, E. S.: Congenital lobar emphysema. Pediatrics 8 795–804, 1951
19 Sh w R. R.: Localised hypertrophic emphysema. Pediatrics, 9 220–227 1952.
20. Sloan H.: Lobar obstructive emphysema in infancy treated by lobectomy J Thor Surg, 26 1–20, 1953.
21 Sweet R. H.: Case records of the Massachusetts general hospital. New England J Med, 242 149–152, 1950.
22. Tveteraas, E.: Congenitalt lobert emfysem. Nord Med 59 742–746, 1958
23. Van Epps, E. F and Davies, D. H.: Lobar emphysema. Amer J Roentgenol 73 375–384, 1955.
24. White-Jones, R. H and Temple, L J Congenital lobar emphysema. Arch Dis Childh 29 49–353, 1954
25. Williams, M H.: Localized pulmonary hypertrophic emphysema. J Thor Surg, 24 522–529, 1952.

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The Effect of Intramuscular Iron-dextran Complex[1] on Iron-deficiency Anaemia in Otherwise Ill Infants and Pre-school Children

by PHILIP LANZKOWSKY[2]

Introduction

This paper describes a trial of intramuscular iron-dextran complex in the treatment of iron-deficiency anaemia in ill Cape Coloured and African[3] infants and pre-school children attending the Out-patient Department of the Red Cross War Memorial Children's Hospital in Cape Town.

Parenteral therapy was especially chosen for treatment of these ill children since this was the only way of ensuring that they received the prescribed dose of iron. It also eliminated frequent hospital visits and its attendant domestic difficulties.

Although oral iron is effective if given continuously *for long periods, parents* may fail for various reasons to administer it regularly and children may not readily accept the medication. The prevalence of acute diarrhoeal disorders (gastroenteritis) amongst under-privileged children is another reason for the employment of parenteral iron, since it cannot be certain to what extent oral iron is absorbed from the gut in the presence of diarrhoea.

Intravenously administered saccharated iron oxide is quite effective in the treatment of iron-deficiency anaemia. The technical difficulty of intravenous injections in small infants and the many recorded untoward reactions are the main disadvantages in its use.

Intramuscular iron-dextran complex, on the other hand is safe effective and easily administered. In infants and children with iron-deficiency anaemia a few workers have shown that iron-dextran complex results in a marked, rapid haematological and clinical improvement and that it is free from any undesirable side-effects (2, 6–19).

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[1] Imferon, Benger Laboratories Limited, England.

[2] Cecil John Adams Memorial Travelling Fellow 1960 and recipient of Dr C. L. Herman Research Grant University of Cape Town Staff Research Fund.

[3] The Cape Coloured people are of mixed ethnic origin and descended from various indigenous and imported groups: Slaves, Hottentots, Bushmen, Bantu and Europeans. The Africans, also referred to as Bantu or Natives consist of various tribes of indigenous groups of Southern Bantu. These people have a poor socio-economic status very much inferior to that of the White people. The African people occupy the lowest socio-economic position, whereas the Cape Coloured community is intermediate between the White and African. Both Cape Coloureds and Africans are ethnically non-White and their social conditions are similar to those of technically less advanced communities.

Present Investigation

Material

This investigation is based on a series of 125 consecutive Cape Coloured and African infants and pre-school children attending the Out-patient Department of the Red Cross War Memorial Children's Hospital in whom a diagnosis of iron-deficiency anaemia was made and who were treated with intramuscular iron-dextran complex. The patients were taken seriatim from those attending the out-patient department for various ailments. Acute diarrhoeal disorder (gastroenteritis) was the commonest illness amongst these infants and children. The majority of illnesses were acute and usually the duration was not more than a few days when the patient was brought to hospital. None presented primarily as cases of anaemia. The fact that these infants and children were out-patient cases does not imply that they were only mildly ill and not needing admission to hospital. There were many cases needing hospitalization but because of shortage of accommodation they could be admitted.

Any out-patient who showed clinical signs of dehydration or who had a blood dyscrasia, for example erythroblastosis foetalis or leukaemia, was excluded. The patients investigated had not received iron other than that present in their usual diet.

The mean weekly family income was £4 1 in the cases of Cape Coloured patients and £3 16 for African patients. This was determined by taking the weekly income of the head of the family in a sample of 50 consecutive patients in the two racial groups. Adopting the criteria of Bronte-Stewart *et al.* (4) and Moodie (13), these figures indicate that the Cape Coloured infants and children were from the "low income" group and the Africans from the "medium income" group in their respective sections of the community.

Of the Cape Coloured and African infants and children 50.3 % and 41.3 % respectively had been breast fed for less than 3 months after birth. This is a much higher proportion than is found in healthy children in this community (11, 12). In general, all these patients had a poor dietary history.

The diagnosis of iron-deficiency anaemia was based on the level of haemoglobin and the morphology of the red blood corpuscles on the blood smear. The critical level of haemoglobin at or below which anaemia was considered to be present and iron medication administered was arbitrarily fixed at 0.5 g % below the lowest figure accepted by Wintrobe (16) as normal for each age group.

Concurrently with the necessary treatment for the presenting illness these infants and children were treated for iron-deficiency anaemia with iron-dextran complex given by deep intramuscular injection. The amount of iron-dextran complex was calculated according to the original haemoglobin level and the body weight[1] and varied from 100 to 400 mg (2–8 ml).

Methods

Blood for haemoglobin was taken from the heel prick of infants and the thumb of older children, using a triangular cutting needle. Free unrestricted flow without necessity for external pressure of any kind was obtained in all cases.

Capillary blood, 0.02 ml, was pipetted into 5 ml of 0.04 % ammonia in water and the haemoglobin level read by the oxyhaemoglobin method using a Klett-Summerson Colorimeter previously calibrated for the purpose against standard haemin and cyanmethaemoglobin solutions. Blood smears were stained by May-Grunewald Giemsa method.

Results

Haemoglobin estimations were made immediately before commencement of iron medication, and at three weeks and six weeks thereafter.

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[1] The formula used was $9W + \frac{W}{6}(100 - \text{Hb }\%)$ = mg of iron,
where W = Weight of patient in pounds
Hb % = Observed haemoglobin percentage
(as recommended by the manufacturers of iron-dextran complex).



Fig. 1 Mean haemoglobin levels in 123 Cape Coloured and African infants and pre-school children before and after treatment with intramuscular iron-dextran complex.

Key: Initial Hb. ——— after 3 weeks — — after 6 weeks ~ ~ Normal value (Wintrobe)

The mean haemoglobin levels before and after treatment with iron are shown in Figure 1 and Table 1. The mean haemoglobin levels before treatment ranged from 8 91 g % to 9 61 g % in the various age groups, at 3 weeks after the injection of iron the mean haemoglobin levels were found to range between 10 12 g % and 10 80 g and at 6 weeks after injection the mean haemoglobin levels had risen further to ranges between 10 75 g % and 1 0 g. The morphology of the red blood corpuscles on the blood smear prior to treatment showed all gradations of an iron-deficiency pattern.

None of the children showed severe untoward reactions after iron administration. Occasionally fever lasting 24 to 48 hours was observed and less commonly localized staining of the skin occurred when there was leakage beneath the skin if the iron was not given deep enough into the muscle or because "streaking" occurred along the path of withdrawal of the needle. This was found to be of no consequence. There was no local tenderness, redness or swelling. The pain during administration, as judged by the reactions of the children, did not appear to be more than that occurring with any other intramuscular injection.

It is clear that, in spite of the presence of an infection there was a statistically significant increase in haemoglobin levels

Present Investigation

Material

This investigation is based on a series of 1.3 consecutive Cape Coloured and African infant and pre-school children attending the Out-patient Department of the Red Cross War Memorial Children Hospital in whom a diagnosis of iron-deficiency anaemia was mad and who were treated with intra muscular iron-dextran complex. The pa tients were taken seriatim from those at tending the out-patient department for various ailments. Acut diarrhoeal disorder (gastroenteritis) was the commonest illness amongst these infant and children. The majority of illnesses were acute and usually the duration was not more than a few days when the patient was brought to hospital. None presented primarily as cases of anaemia. The fact that these infants and hildren were out patient cases does not imply that they were only mildly ill and not needing dmission to hospital. There were many needing hospitalization but because of shortage f accommodation they could be admitted

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Concurrently with the necessary treatment for the presenting illness these infants and children were treated for iron-deficiency anaemia with iron-dextran complex given by deep intramuscular injection. The amount of iron-dextran complex was calculated according to the original haemoglobin level and the body weight[1] and varied from 100 to 400 mg (2–8 ml)

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Results

Haemoglobin estimations were made immediately before commencement of iron medication and at three weeks and six weeks thereafter

[1] The formula used was $9W + \frac{W}{4}(100 - Hb\ )$ = mg of iron
where W = Weight of patient in pounds
Hb = Observed haemoglobin percentage
(as recommended by the manufacturers of iron dextran complex).

Discussion

After intramuscular administration of iron-dextran complex the rate of recovery from iron-deficiency anaemia depends on several factors of which the most important are the presence and severity of the associated infection, the type of tissue into which the iron is injected, the mobilization of the limb and the initial degree of iron shortage

The anaemic sick infants and children in the present investigation responded well to intramuscular iron-dextran complex. The presence of acute infection, and of some infections which were of longer duration, did not prevent a significant increase in haemoglobin level The duration of what has been called "acute infection" cannot be accurately and definitely determined but generally was not more than a few days. Although they were all out-patients they were not all mildly ill. In deed, an appreciable number of the patients were severely ill

Absorption of intramuscular iron-dextran complex may be diminished if a proportion of the dose becomes trapped in the subcutaneous fat (8) The evidence of this is gross skin-staining after injections In obese subjects particularly some of the dose may enter fatty tissue (16) This is really due to faulty injection technique Since the majority of the present subjects were undernourished, many of them grossly so the factor of obesity need not be discussed any further

Another factor influencing absorption is the mobilization of the limb following intramuscular injection Grimes & Hutt (8) stated that four of their patients were confined to bed at the time of the injection, and the least mobile showed the lowest eventual clearance They concluded that exercise of the limb after injection increases absorption, and that in obese immobile patients one should administer a dose slightly higher than the calculated requirement of iron.

All the subjects of the present study were out patients. It is reasonable to assume that all those over one year of age were more than ordinarily active since for example their economic circumstances would force them to walk where children in better circumstances are transported.

In the investigation done by Wallerstein & Hoag (17) the magnitude of the haemoglobin response varied with the degree of anaemia The haemoglobin level in some of the children with severe anaemia increased by 4 % per day during the first week of treatment while others with less marked anaemia showed little or no measurable rise during that time. In the present investigation as well, the response to intramuscular iron-dextran appeared to vary inversely with the initial haemoglobin level.

It was the author's impression that improvement in appetite and diminished irritability after intramuscular iron-dextran complex appeared before any rise in haemoglobin level was demonstrable This rapid response was also observed by Yu *et al* (19) Beutler's (3) view is that part of the symptoms of iron-deficiency anaemia may be related to deprivation of tissue iron enzyme and not chiefly to the anaemia He deduced this by showing a marked decrease in the cytochrome c content of the liver and kidneys in iron-deficient rats. This decrease greatly exceeded the slight decrease in haemoglobin concentration.

The total calculated dose of iron dextran complex in this investigation was given at the initial consultation This was done in order to reduce the number of hospital visits which in a poor community entails considerable financial and domestic hardship Despite these very large doses no severe untoward local or systemic effects were observed. It should not however be assumed that there is no limit to the amount which can be given.

Prior to the commencement of administration of parenteral iron consideration was given to the possibility of producing *haemosiderosis or haemachromatosis*. Since there is no effective mechanism for iron excretion, injected iron is almost entirely retained and excessive therapy might lead to iron overload Yu *et al.* (19) have stated that humans cannot excrete more than 1 mg of iron in 24 hours. There a wide margin of safety however and at least 1 g of intramuscular iron-dextran complex may be given to infants with impunity (17) The amount of iron needed to produce haemosiderosis or haemachromatosis is *approximately 25 g* As the maximum therapeutic dose in the adult is 5 g there is a five fold safety factor (5) In infants receiving 100 mg to 400 mg the safety factor is probably much higher One would not expect this to be a problem provided that the proper dosage is used and a safe maximum is not exceeded. No evidence of a detrimental effect in the children of the present investigation has so far appeared Nevertheless, it must be strongly emphasized that failure of a patient to respond to the proper intramuscular dose of iron is never an indication for giving more iron

This is important especially in the light of recent research work which has shown that iron-dextran complex in massive doses may result in excessive deposition of iron in the interstitial cells of the testes and testicular atrophy in mice (14) and that it may induce sarcoma at the site of injection in rats and mice (9 10 15) The dosage of iron-dextran complex used by Richmond (15) was massive—some 200- to 300-fold greater than the therapeutic dose and that used by Haddow & Horning (10) represented in relation to body iron, a swamping dose, both locally and systemically The clinical dose for *mice would be only 1/100th or 1/150th* of that used in Haddow & Horning's experiment (10) In the lower dose used by Richmond (15) the incidence of tumours dropped sharply to become comparable with, or even lower than, published figures for tumours obtained in a similar manner by repeated injections in rats and mice of glucose, fructose, arachis oil, and many other innocuous materials (7)

Apart from the important dosage factor repeated trauma muscle necrosis and chronic *inflammatory changes caused* by repeated injection into the unstable subcutaneous tissues of rats and mice can lead to the production of sarcomata (7) Sarcomata following injection of iron dextran complex have been produced with any degree of frequency only in rats and mice Rat connective tissue cell are notoriously susceptible in responding with sarcomas to injection of the most diverse agents (1) Other species which have received massive over-doses of iron-dextran, for example, the rabbit and guinea pig did not develop tumours (10)

An assessment of the clinical significance and implication of these observa

tions is not possible at present and will require an elaborate study of the many factors involved.

Summary

1 One hundred and twenty five ill infants and children who were found to have iron deficiency anaemia were treated with intramuscular iron-dextran complex with doses varying from 100 to 400 mg, concurrent with treatment for the presenting illness.

2 The mean haemoglobin levels before treatment ranged from 8.91 g % to 9.61 g % in the various age groups; at 3 weeks after the injection of iron the mean haemoglobin levels were found to range between 10.12 g % and 10.80 g % and at 6 weeks after injection the mean haemoglobin levels had risen further to range between 10.75 g % and 12.0 g %. There was thus an approximate general rise of g % of haemoglobin in 6 weeks. Clinically the beneficial effects were quite striking, and occurred often within days.

3 The results of this investigation indicate that intramuscular iron-dextran complex can significantly raise the haemoglobin level in infants and children who have iron-deficiency anaemia with a variety of associated illnesses of all degrees of severity and of largely undetermined duration. In addition, iron-dextran complex was found to be safe and easy to administer.

4 The factors influencing rate of recovery from iron-deficiency anaemia after intramuscular iron-dextran complex, the theoretical dangers of iron overload and the possible production of sarcomata in iron injected tissue are discussed.

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References

1 Arnott, ... Ten Years with Tumour Viruses. *Lancet* 2 237 1957
2 Bartlett W H and Beatty E. C The treatment of iron deficiency anaemia in children with iron dextran. *Amer J Dis Child* 94 661 1957
3 Beutler, E Iron enzymes in iron deficiency I Cytochrome C *Amer J Med Sc* 234 517 1957
4 Bronte ... B ... A., Brock, J F Moodie, A D., Keys, M H. and Antonis, A Serum cholesterol diet and coronary heart disease an inter racial survey in the Cape Peninsula. *Lancet* 2 1103 1955
5 ... D H ... A R J and ... C A Treatment of iron deficiency anaemia *Blood* 10 567 1955
6 G ... D. and Jennison R. F Intramuscular iron in infancy *Brit Med J* 2 700, 1955.
7 Goldberg L. Imferon Letter to editor *Brit Med J* 1 954, 1960.
8 Grimes, A J and Hutt M. S R.: Metabolism of 59 F -dextran complex in human subjects. *Brit Med J* 2 1074, 1957
9 Haddow A. (1958) (Quoted by Richmond, H G) Personal communication.
10 H ... A and Horning, E. S On the carcinogenicity of an iron dextran complex *J Nat Cancer Inst.* 24 109 1960.
11 Lanzkowsky P Iron deficiency anaemia in infants and pre-school children in three racial groups in Cape Town M D Thesis, University of Cape Town, 1959

12. — Mean haematological values in healthy infants and pre-school children in Cape Town. *S Afr Med J* *34* 489 1960.
13. Moodie, A. D.: Personal communication, 1959
14. Nissim, J. A.: Deposition of iron in testes after administration of iron-dextran complex. *Lancet*, *1* 01 1953.
15. Richmond H. G. Induction of sarcoma in the rat by iron-dextran complex. *Brit Med J* *1* 947 1959
16. Scott J. M.: Intramuscular iron therapy in anaemia of pregnancy- year survey. *Brit Med J* *2* 635, 1956.
17. Wallerstein R. O. and Hoag, M. S.: Treatment with iron-dextran of iron-deficiency anaemia in children. Paper read before the 10th Clinical Meeting of the *Amer Med Ass* *Seattle*, Nov 30th, 1956.
18. Wintrobe, M. M.: Normal values for number of erythrocytes, quantity of haemoglobin and volume of packed red cells. Clinical Haematology 4th ed. Philadelphia, Lea and Febiger 1956, p 100.
19. Yu W. L., Mahoomian, K. and Watson R. J.: Comparison of intramuscular iron dextran and oral ferrous sulfate in the treatment of iron-deficiency anaemia. *J Pediat*, *54* 50, 1959.

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A Study of Group A Hemolytic Streptococcus Carriers Among School Children

Part II Significance of the Findings[1]

by P MOZZICONACCI, CL. GERBEAUX, R. CARAVANO S GERBEAUX, J LABONDE, S. RAHMAN, F RABCYNSKA, E. ORSSAUD and P VIROLLEAU

Discussion

The results which we obtained in the course of the study are of two kinds those which are of bacteriological interest, and those which chiefly concern the epidemiology of the hemolytic streptococcus in healthy subjects.

1—*Bacteriological study*

One of the aims of our study was to develop a technique of taking throat swabs and of their subsequent culture which would be as sensitive as possible and suited to the needs of our epidemiological survey

Between the taking of the throat-swab and the grouping of the organism, a number of difficulties and possible causes of error may arise These may affect the following areas the material used for the throat swab and the way in which it is taken, the transport of the swab isolation (with or without the use of a selective medium) the reading of the blood-agar plates; finally the culture medium used for the re-seeding of suspect colonies

A—Material technique and transport of swabs In the course of meetings of the Committee on Acute Respiratory Diseases in 1946 and 1948 (3) Wannamaker *et al* (25) showed that isolation should be done on the spot so as to avoid the risk of the throat swab drying up. Rubbo & Benjamin (1) have demonstrated that cotton wool rapidly kills the organism and that the *survival of the streptococcus* in the throat swab will increase from 2 days to 10 if the carded cotton used is dipped in clotted serum. In a general way all the authors have advised against the transport of the throat swab as such in a dry state this has led Pike (14) Wannamaker (24) Holmes (9) and Werner & coll. (26) to use selective media for the detection of healthy carriers the same has been the case for the detection of carriers during streptococcal infections or in rheumatic fever (Feldman & Harmon (6))

For our part, we endeavoured to eliminate these causes of errors by performing all the initial manipulations on the spot.

[1] Part I was published in the November issue of Acta Pædiatrica 1960

TABLE 8.

Authors Date	No. of swabbings or of A found	% of A found without medium	with selective medium
Pike—1945	131	19	81
Wannamaker & coll. 1948	330 A	31.2	61.9
Feldman and Harmon, 1952	103 A	20	26
Holmes—1956	89 subjects	68.4	81.6
Werner—1958	78 A	60	69

(1) when the swab was being studied without the use of a selective medium the blood-agar plates were brought to the place where throat swabbing sessions were being held and isolation was performed then and there. (2) when we used selective media, the tubes were brought to the place of swabbing and the throat swab was immediately introduced into the fluid or semi-solid medium

B—*Direct isolation and isolation after passage through a selective medium.* Pike (13) demonstrated that crystal violet and sodium azide added to broth favour the growth of hemolytic streptococci while inhibiting that of associated germs. The subsequent studies of Wannamaker (24) Feldman & Harmon (6) Holmes (9) and Werner (26) confirmed his conclusions. Holmes (9) wishing to devise a suitable transport medium, modified the concentrations of sodium azide and crystal violet *given by Pike and added agar-agar to* render it semi-solid. The comparative results obtained by these authors are summed up in Table 8

On the other hand, these authors were able to elucidate a number of points concerning isolation proper whether direct isolation or isolation after passage through a selective medium. Thus, Wannamaker & coll. demonstrated (25) that more positive swabs are found in healthy carriers if as many suspect colonies as possible are taken from a single plate and if several plates are seeded with the same throat-swab.

Fry (8) and Holmes (10) have emphasized the favourable effect of anaerobiosis, and Werner & coll. of mass seeding (26)

Under the working conditions of our laboratory it was not possible in 1957–1966 to seed several plates with the same throat-swab An attempt was made to reseed *as many suspect colonies as possible* and we made a special point of studying and developing an efficacious selective medium This led to the study of 3 media all of which were based on a meat extract broth buffered at pH 7.8 they differed in their concentration of crystal violet and sodium azide

During the year 1957–1958 3 media were thus investigated comparatively in *direct isolation on blood-agar* The negative results obtained with the first of these media (Technique 2) soon led to the abandonment of these concentrations of sodium azide and crystal violet and to the adoption of a medium rather similar to that proposed by Holmes.

This technique enabled us to demonstrate up to 10 times as many group A strepto-

TABLE 9–11 *Direct smears and fluid medium adapted from Pike*

TABLE 9

	Basic medium	Concentration and added products	No. of swabs	No. of A	% of A
Technique 1	—	—	218	7	3.2
Technique 2	Peptoned, glucosed meat extract broth pH 7.8	NaN_3 1/400,000 Crystal violet 1/500 Defibrinated blood 3/100	193	0	0

TABLE 10

	Basic medium	Concentration and added products	N of swabs	No. of A	% of A
Technique 1	—	—	663	20	3.1
Technique 3	—	NaN_3 1/1,000 000 Crystal violet 1/16,000 Agar-agar 15/1000	570	125	21.9

TABLE 11

	Basic medium	Concentration and added products	N of swabs	No. of A	% of A
Technique 3	—	NaN_3 1/1,000,000 Crystal violet 1/16,000	191	21	10.9
Technique 4	—	NaN_3 1/1,000,000 Crystal violet 1/16,000 Autolysate 2/1000	237	38	16

cocci as with direct isolation on a blood agar plate. Contrary to the opinion of most authors who have used Pike medium (especially Feldman (66)) we have not observed that our semisolid medium derived from that of Holmes particularly favoured streptococci of Groups B, C and G. On the contrary, as regards Group C and G streptococci, we could not obtain an increase as great as that of Group A.

During the year 1958 it was this medium derived from that of Holmes, which was used for purposes of comparison. In the 3rd medium used, the concentrations of sodium azide and crystal violet were not modified, the agar-agar was omitted so as to facilitate a better dispersion of the organisms and some barm autolysate was added to increase the growth of the streptococcus even more.

This technique did not improve our results as strikingly as did Technique 3 when compared with direct seeding; nevertheless, this medium (Technique 4) enabled

TABLE 12

No. of carriers	13	25	20	17	15	11	15	13	14	10	9	11	7	18	18	9	9	9	10
No. of children	83	84	85	87	85	71	84	87	84	86	87	45	47	88	89	85	[illegible]	[illegible]	[illegible]

$\chi^2 = 34.5$ with $V = 18$ degrees of freedom $P = 0.01$

TABLE 13

Survey	Year	Number of cases	Maximum %
Bourn	1932	2 812	N relationship
Hantz	1941	643	Winter and early spring
Riley	1953–1955	175	N relationship
Baslaw	1953	343	May
	1953–1954	417	Febr./Apr May
	1954–1955	331	F br./March
	1954–1955 Special study		March
Cornfeld	1955–1956		Jan./April, May
	School W	468	
	School B	456	Jan., May
	1956–1957		
	School W	454	January
	School B	285	April

us to further increase the rate of our carriers in a marked and statistically significant way Tables 9 10 and 11 sum up the results obtained.

We believe therefore that, for the time being medium 4 is the most sensitive and the most suitable for epidemiological studies of the type that we have undertaken.

II—*Epidemiological study*

A—*Variations in the percentage of carriers* We have seen (Fig 1 cf Part I) that the percentage of carriers varies during the course of the year This variation is statistically significant i.e within the limits of precision of the method used, it is not due to chance fluctuations among a population with a constant rate of carriers (Table 1)

Most of the authors who have performed regular throat swabbings have noted similar variations which they were naturally inclined to ascribe to seasonal factors (Table 13)

These studies are difficult to compare because of differences in the techniques used. It is however generally agreed that streptococcal morbidity reaches a peak at the end of winter and in early spring It has been seen that most authors consider this to be true for carriers as well. Our own observations would seem to lead to the same conclusion. It should be pointed out, however that several theoretical conditions must be fulfilled *before* variations in carrier percentages can be linked in an unquestionable way with seasonal influences. The variation around the average must not be due to chance We have seen that this condition has been fulfilled in the case of our results. The curve

of seasonal variations should reproduce itself regularly from year to year and for several years running. The latter condition is much more difficult to abide by. It is in fact, impossible to consider following a population for some ten years with the sole purpose of verifying the existence of an annual peak; in addition, the changes likely to occur during that period in the population studied would restrict the meaning of the observations; lastly, changes in microbial diffusion over the years would make the conclusions reached only relative.

However the remarkable agreement of several of the studies referred to above added to our own would give us reason to believe that there is an annual peak of the percentage of carriers in early spring. It should be noted in this connection that the four concording studies (Rantz (19), Saslow (23), Cornfeld (4) and ourselves) have borne on different populations followed up at different periods (1941, 1953 to 1958) in different regions (all situated, however, in the temperate regions of the northern hemisphere). This divergency of conditions to our mind makes the similarity of the results all the more significant.

A third condition is the demonstration of a direct relationship between the cyclical variation and a measurable seasonal factor. The definition of a season is of course difficult to express in figures although it is current practice to note a change of season objectively. We attempted to verify the existence of a correlation between the percentage of carriers and certain meteorological factors. We found that there was no detectable relationship between this percentage and the average temperature, rainfall, barometric pressure, or the total number of hours of sunlight.

There apparently exists a correlation with average humidity although of a type that would seem contrary to generally accepted ideas. Indeed, our percentage of carriers varies considerably in inverse ratio to the percentage of average humidity. We believe, however, that this relationship is not a real one and that it is due to the fact that in the region investigated, the relative humidity shows a marked decrease from the month of March onwards.

The question of seasonal variations and of the factors that bring them about is still open. Strictly standardized observations are required before it may be regarded as having been finally resolved.

B—*The problem of the carrier factor.* When it is noted, as mentioned above, that the carriers can be fairly easily divided into two groups: casual carriers and prolonged carriers, the question naturally arises whether this difference of behaviour is due to a specific factor. The problem may be approached in different ways. In this study, we have attempted to solve it by means of the statistical method. If no special factor is involved influencing the distribution of carriers, the latter is purely a matter of chance. It can then be compared to a dealing out of a pack of playing cards, where the chances any subject has of receiving the "streptococcus card" can be calculated for each deal, since we know each time the probability of distribution of these streptococcus cards.[1]

[1] This probability depends on the percentage of carriers following each swabbing, i.e. it varies from one deal to another and must be recalculated each time.

TABLE 14

	Children with tonsils		Children tonsillectomised		
	No.	%	No.	%	Total
Non carrier	13	52	12	48	25
Carrier 1 to 4 times	19	70	8	30	27
Carrier more than 4 times	17	89	2	11	19
Total	49		22		71

$\chi^2 = 7.2$ with $3 - 1 = 2$ degrees of freedom. $P < 0.05$.

In these circumstances the theoretical cumulative percentage of carrier subjects can also be determined After a certain number of deals it should, of course, tend to 100 %, the chances being equal for all the subjects to receive the streptococcus card" at a given time.

To bring this point out more clearly we tried to select as homogeneous a group as possible out of our total population. *Statistical calculation having shown that* absence from more than 5 swabbings created a factor of heterogeneity we discounted all subjects who had been absent 5 times or more during the study We were thus left with a more restricted but a more homogeneous sampling of 89 children.

Figure 6 (cf. Part I) opposes the theoretical curve calculated in this way and the real curve which we have observed. It will be easily seen that the latter rises rapidly at first but then stays at a level much below the theoretical maximum It is therefore permissible to infer that the distribution of the "streptococcus card is not due to chance and that consequently there is a systematic factor connected either with the existence in the prolonged carrier of a special facility for acquiring the organism, or to the existence in the habitual non carrier of a special defence against the same organism

The real nature of this factor cannot, of course be ascertained by the same method, this can only be done by biological investigation.

C—*Biological and sociological factors connected with the carrier state.* Thinking our belief in the existence of a carrier factor to be *justifiable we attempted, by means* of a medico-social questionnaire of each of the children observed, to determine the correlations that might exist between the carrier state and the physiological and environmental conditions of the subject.

The investigations were made in the same group of subjects as above, and for statistical reasons we considered as prolonged carriers the children whose throat swabs were A positive 5 times or more during the survey

We were thus able to demonstrate 3 definite correlations:

(1) Tonsillectomized subjects are less often prolonged carriers than those who still have their tonsils (Table 14) This idea has already been generally accepted and with the exception of Quinn & Riley (17) all the

TABLE 15

	No running water		Running water		
	No.	%	No.	%	Total
Non carrier	1	4	24	96	25
Carrier 1 to 4 times	7 } 13	28	20 } 34	74	27 } 46
Carrier more than 4 times	6		14		19
Total	13		58		71

$\chi^2 = 5.4$ with $F = 1$ degree of freedom. $P = 0.02$.

TABLE 16

	Brown eyes		Blue eyes		
	N	%	No	%	Total
Non carrier	7	28	18	72	25
Carrier 1 to 4 times	17	63	10	37	27
Carrier more than 4 times	12	63	7	37	19
Total	36		35		71

$\chi^2 = 6.0$ with $F = 2$ degrees of freedom. $P = 0.05$.

authors who have undertaken a similar study have reported such a correlation (5 7 11 15 18 77)

(2) The environmental health conditions of the carrier are also a well known *factor*. We have found a strong correlation between the prolonged carrier state and a particular factor of hygiene namely the absence of running water in the dwelling house (Table 15)

It is evident that the absence of running water in an apartment nowadays indicates a very low standard of sanitation especially in an urban environment. It is however interesting to note that we have recorded no other correlations between the prolonged carrier state and other criteria of hygiene such as those connected with the situation of the dwelling house (lighting, ventilation, dampness) amenities such as heating, gas, electricity or the conditions of accommodation; as regards the latter point, it is to be reported that we have found no correlation between the carrier state and overcrowded lodgings.

(3) As regards genetic factors, it is generally asserted that heredity plays a part in certain complications of streptococcal infection, in particular rheumatic fever. But although widely accepted, this view has not been established on the strength of any very sound observations, with the exception of some recent studies which report that rheumatic subjects belong to certain blood groups more often than controls.

Be that as it may, the predisposition to streptococcal infection and to its complications (especially rheumatic fever) is a very different thing from the predisposition

TABLE 17

	Children with tonsils		Children tonsillectomized		
	No.	%	No.	%	Total
Running water	36	62	22	38	58
No running water	13	100	0	0	13
Total	49		22		71

$\chi^2 = 7.07$ with $V = 1$ degree of freedom. $P < 0.01$

TABLE 18.

	Brown eyes		Blue eyes		
	No.	%	No.	%	Total
Children with tonsils	27	55	22	45	49
Children tonsillectomized	9	41	13	59	22
Total	36		35		71

TABLE 19

	Brown eyes		Blue eyes		
	No.	%	No.	%	Total
Running water	30	52	28	48	58
No running water	6	46	7	54	13
Total	36		35		1

to being a transient or chronic carrier which is what interests us at the moment. Our study demonstrates a definite correlation between the carrier state and a genetically controlled factor viz. the dark colour of the iris (Table 16). To our knowledge this is the first time that a correlation of this kind has been reported in this field. It has been verified that this correlation is neither associated with a common ethnic origin nor with the fact of belonging to the same residential area.

There is a partial correlation between the presence of running water in the apartment and tonsillectomy (Table 17). On the contrary, the correlations between the colour of the iris and tonsillectomy (Table 18) or the running water (Table 19) are unconnected. they are therefore independently significant.

For other physiological or sociological factors we have found slight connections which do not allow of unqualified conclusions. A new survey is in progress, one

of the aims of which is precisely to attempt to bring out the probability of these correlations more clearly [1]

III—*The limits of interpretation*

We have raised the question of the extent to which the considerable inadequacies of our technique of bacterial research, as shown by the error calculation set forth above, might limit the scope of the conclusions which we believe to have reached, especially as regards the existence of prolonged carriers, for this problem seems to be in the forefront nowadays.

It is evident that since the results of swabbing performed in identical conditions, vary markedly with the technique used, questions of technique greatly modify the value that can be ascribed to them.

The manipulations which lead to the final identification of a Group A streptococcus in a throat swab are many and their importance for the reliability of the ultimate result varies. We have set forth above, in the course of the discussion of our technical results several points that the experience of other authors and ourselves permitted us to elucidate but it must be admitted that a searching methodological study *has not yet been made*

It can be seen that the technique on the results of which our figures are based (Technique 3) fails us once every 3 times (34.5 % of failures). No categorical value can therefore be attached to a single negative swab either for concluding that a child is not a carrier or for asserting that he has ceased to be one.

On the other hand, when a subject hitherto negative happens to present a positive swab at a given time we are unable to claim that this is a recent acquisition of the organism since Table 7 clearly brings out the fact that the same strain can be found in a subject after a long period of apparent absence

The imperfections of the method lead us therefore to results falsified by default on the one hand, since we regard as negative the subjects who have one chance in three of being carriers, in fact, and by excess, on the other if we consider as an acquisition the reappearance in a subject of a strain which had been lost for a time due to an inadequate technique.

It is, of course impossible to guard against the first of these errors. The second can, however be eliminated by considering as an acquisition in a given subject only the appearance of a new type of strain. As we had no regular typing of all our strains we have been unable to use this method and, this being so we have decided to base our argument on the cumulative incidence

[1] The following correlations with the state of being "prolonged carrier" are studied statistically Hair; Eyes; Complexion; Twins, Congenital malformations, Age of the father; Age of the mother; Siblings; Weight Height; Puberty; Personal cleanliness General condition, Cuti-reaction—positive due t BCG Streptococcal disease; Surgical operations, Hospitalisation; Kind of dwelling-place Floor inhabited, Exposure to sunshine; Ventilation; Humidity; Heating, W C Water supply Hygiene conditions, Electricity N of persons living at home; No of rooms; Presence of the father t home; Allergy in the father Streptococcal disease in the father; Father profession; Father' conditions of ork Conditions of hygiene of the same; Presence of the mother at home; Allergy in the mother Streptococcal disease in the mother; Mother profession; Mother' conditions of work, Conditions of hygiene of the same; Animals at home Outside relationships of the child, Sex of the parents Travel and stays out of town; Allergy in the 1st 2nd, 3rd and 4th child; Streptococcal disease in the 1st 2nd, 3rd, and 4th child

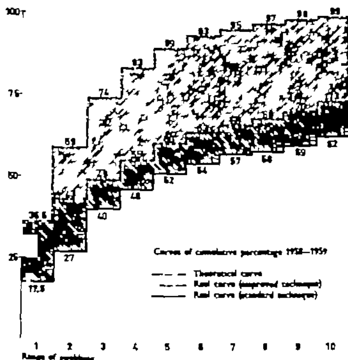


Fig 8.

of the carriers every subject being considered as a new carrier only the first time that a swab of his is found to be positive, whatever may be the result of subsequent swabbings

Under these conditions we believe that the interpretation based on Fig 6 (cf Part I) remains valid, especially if it is considered that the real curve differs even more from the theoretical curve when it is corrected by taking the technique into account It is seen in Fig 5 (cf Part I) that the number of new carriers is considerable especially following the first two swabbings; this seems to us to be due to the fact that the technique being imperfect two samplings are necessary in order to obtain the value of the average carrier percentage *around which the variations* will eventually be much smaller

This conclusion seems to have been confirmed by the first results of another study now under way Another group of children has been under study for 8 months in conditions rather similar to those of the above study but with a technical improvement which ensures more regular results. This new technique has in particular the effect of diminishing the number of false negatives, a fact which can be noted by studying the continuity of positive swabs in the carriers.

Figure 8 shows the results of the first 10 swabbings performed under these new conditions. It superimposes 3 curves: the theoretical curve of distribution according to chance the curve observed with the standard technique and that obtained with the *improved* technique. It will be seen that the latter while yielding a larger

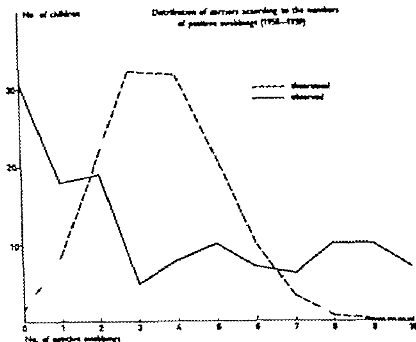


Fig. 9

number of positive results as compared with the standard technique does not produce a curve differing greatly from that given by the latter and that, although it differs notably during the first swabbings (a fact which confirms the argument set forth above) this divergence diminishes with accumulating results. While the lower hatched zone does represent a technical gain, there still remains a fundamental difference represented by the upper hatched zone between the theoretical distribution curve and the curve actually observed. And this difference demonstrates the presence of a carrier factor (or an anti-carrier factor).

It may, of course, be argued that an even finer technique would allow of further increasing the number of carriers. This is very likely, but it seems to us that in view of the very different shapes of the real and the theoretical curves and the fact that this difference is further enhanced by technical improvements (Fig. 8) such a purely technically obtained increase in the percentage of carriers would not succeed in altogether eliminating the class of subjects refractory to the carrier state.

Figure 9, constructed with the data of the improved technique shows clearly that the dispersion of carriers once, twice, three times, or n times is fundamentally different from a dispersion due to chance even when experimental error is greatly reduced.

We therefore feel justified in concluding that the interpretation of our findings demonstrates in the course of a prolonged study 4 types of behaviour with regard to

group A streptococci in a population of normal children: subjects who are never carriers, subjects who are occasional carriers, prolonged carriers of the same strain, prolonged carriers of frequently changing strains.

The limitations of the method seem to be of another kind and on this point our opinion is similar to that of the authors who have recently studied the subject (Saslow (23) Cornfeld & coll. (4)). These differences in behaviour of the subjects with regard to streptococci cannot now be investigated further simply by the detection of the organism in the throat, however sensitive the technique used. They probably correspond to constitutional differences in immunity for the study of which another method must be developed.

It is possible that the different techniques of organism detection may demon e different classes of carriers: it may example be considered that the carriers discovered with the coarsest techniques are those who harbour organisms of greater vitality or in larger numbers. But this is a hypothesis which in any case would have to be confirmed by a parallel investigation. We believe for our part that one way of approaching the problem, as it arises now, would be to make a searching study of streptococcal immunity: it is this approach that has oriented our present investigations.

Summary

In the course of a study pursued for 2 consecutive years in normal children of a suburban public school of the Paris area, the following findings were noted. (1) when the technique used was sufficiently sensitive, the rate of healthy carriers was relatively high, 16.7 % on an average with a range of 6.7 % to 30 %. (*) this percentage seemed to present variations during the course of the year which are perhaps due to a seasonal factor. (3) the cumulative percentage was such that at the end of two years more than one subject in two was found, to have been a carrier of hemolytic streptococci at least once. (4) there was no correlation between the rate of carriers and the development of clinical infections (septic sore throat, scarlet fever). (5) the subjects fell into 4 groups: refractory subjects, occasional carriers, prolonged carriers of the same strain, prolonged carriers of different strains. (6) a prolonged carrier factor was found to exist statistically i.e. certain subjects were particularly receptive to the Group A hemolytic streptococcus or conversely certain subjects were refractory. (7) within the scope of our study this factor was found together with various other independent correlations. Of these, the correlations with the presence of tonsils and with bad conditions of hygiene (absence of running water in the child's home) were hardly surprising; that with a genetic factor (dark iris) was more unexpected, but has been established on equally as sound a basis.

Erratum

On Fig. 6 and Fig. 8, the curves have been constructed with the total number of carriers instead of the cumulative percentages. This, however, makes no significant difference in the shape of the curves.

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References

1. Bouche J M., Carpenter, H. M. and Mc Coma E. Streptococcus haemolyticus in the presence and absence of acute minor respiratory infection 1928–1931 Amer J Hyg 17 41, 1933.
2. Bunn W H. and Bennett H. N Community control of rheumatic fever J.A.M.A. 147 986–989 1955.
3. Committee on Acute Respiratory Diseases. Problem in determining the bacterial flora of the pharynx. Proc Soc Exp Biol Med, 69 45 1948
4. Cornfield D Wheeler, G., Hubbard J P Weaver, R. and Bellows, M.: Streptococcal infection in school population. Ann Intern Med, 49 1305 1958.
5. MacDonald J Simmons, R. T and Krogh, E. V Further observations on the incidence of hemolytic streptococci in the tonsils. Med J Aust 1 849 1940
6. Feldman H. A and Harrison W Comparative effectiveness of direct blood agar and modified Pike broth for demonstrating β hemolytic streptococci of different serological groups. J Lab Cl Med 40 297 1952
7. Frisch A. W Haemolytic streptococci from the throat of normal young adults. J Infect Dis 62 40, 1938.
8. Fry R. M An anaerobic method for identification of hemolytic streptococci J Path Bact 37 337 1933.
9. Holmes M C and Lermitt A Transport and enrichment media. Monthly Bull M inst Health (Lond) 14 97 1955
10. Holmes, M. C and Williams R E O The distribution of carriers of streptococcus pyogenes among 2413 healthy children J Hyg (Lond) 52 165, 1954
11. Krogh, E. V MacDonald J Boyle, G and Simmons, R T The serological group and types of streptococci isolated from excised tonsils Brit Med J 2 1036 1939
12. Lancefield R. C.: The antigenic complex of haemolytic streptococcus. J Exp Med, 47 91 1928.
13. Pike, R. M.: An enrichment broth for isolating hemolytic streptococci from throat swabs. Proc Soc Exp Biol Med, 57 186, 1944.
14. Pike, R. M.: The isolation of β hemolytic streptococci from throat swabs. Amer J Hyg 41 211 1945
15. Pike, R. M. and Fasckeka, G T.: Frequency of streptococci in the throats of well children in Dallas. Amer J Public Health, 36 611, 1946
16. Poskanzer, D C. et al Epidemiology of civilian streptococcal outbreaks before and after penicillin prophylaxis. Amer J Public Health 46 1513, 1956.
17. Quinn R. W Denny F W and Riley H. D Natural occurrence of hemolytic streptococci in normal school children. Amer J Public Health 47 995 1957
18. Rammelkamp C H Jr Streptococcal Infection. Edited by Maclyn McCarty—Columbia Univ Press, New York 1954
19. Rantz, L. A.: The haemolytic streptococci. Studies on the carrier state in the San Francisco area, with a note on methods of isolation and serological identification of these organisms. J Infect Dis 69 248, 1941.
20. Riley H. D Quinn R. W and Denny F W Streptococcal carrier studies in children. Amer J Dis Child 93 84, 193
21. Rubbo, S D. and Benjamin M. Some observations on survival of pathogenic bacteria on cotton wool swabs Brit Med J 1 983 1951
22. Rumpf, E. L.: Control of streptococcal throat infections in schools. A cooperative program followed in Orange County Calif Med 85 385, 1956.
23. Saslaw M. S and Streitfeld, M (1952, 1953–54, 1954–55) Group A β hemolytic

streptococci in relation to rheumatic fever. Amer J Dis Child, 92 550, 1956.
24. WANNAMAKER, L. W.: American Heart Assoc. Pamphlet N Y 1956.
25. WANNAMAKER, L. W et al. The single throat culture as an index of the bacterial flora of the respiratory tract. Amer J Hyg 64 158, 1949
26. WERNER, G., CORNFIELD D., HUBBARD J P and RAKE, G.: A study of streptococcal infection in a school population laboratory methodology Ann Intern Med, 49 1320 1958.
27. WHEELER, S M., JONES, T D.: Factors in the control and spread of acute respiratory infection with reference to streptococcal illness and acute rheumatic fever. Amer J Med Sci, 53 209 1945.

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(Head: Professor Oluf Andersen)

The Incidence of Head Injuries in Children

by JOHANNES C. MELCHIOR

In paediatric departments and, in particular departments to which many patients are admitted with neurological and psychiatric disorders, information is frequently obtained in the case history that the patient has suffered from a head injury. The question arises: What is the value of this information and, just as important, how frequently does head injury occur in general in children? Only isolated numerical reports concerning the incidence of head injury in childhood are available and these are usually concerned with special categories of patients suffering from neurological and/or psychiatric symptoms.

Dyggve in psychiatric material of children obtained only scanty information concerning head injury. Otto from among 3585 children seen in psychiatric advisory clinics in Stockholm found that 20 per cent of all the patients had a history of head injury and that in $\frac{1}{3}$ of the cases the injury had been associated with loss of consciousness. In this material boys were 3–4 times as common as girls. Melchior in a follow-up investigation of children from a department of neurosurgery found that 24 per cent of all the histories contained information about head injury.

It appears to be of interest, particularly in the evaluation of head injuries in neuro-psychiatric patient material, to determine the incidence of head injury among children without any such symptoms.

Present Investigations

In order to obtain information concerning the incidence of head injury in children other than neurological and/or psychiatric patients, inquiries were made regarding head injury in all patients admitted to hospital over a period of eight months. The question: Has the child ever had a head injury? was put to the parents. If the reply was in the affirmative, inquiries were made concerning symptoms such as headache, vomiting, vertigo, loss of consciousness, whether a doctor was called, and treatment in a casualty department or admission to hospital if this occurred. During the eight month period, 1190 patients were admitted and in 56 cases the desired information was not obtained. In some cases this was due to the fact that there was simply no time to undertake routine questioning as the child concerned was desperately ill or for some other reason.

As already mentioned it may be assumed that in a department which receives many children with neurological conditions (cerebral palsies, epilepsy, mental retardation etc.), information concerning head injury will occur more frequently in the case his-

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TABLE 1 *Occurrence of history of head injury among children admitted with or without neuropsychiatric symptoms*

	Girls				Boys			
Number admitted	471				663			
Neuro-psychiatric symtoms	− 377		+ 94		− 517		+ 146	
History of head injury	N	Yes	N	Yes	N	Yes	N	Yes
Number	337	40	65	29	448	69	106	[illegible]

tories and therefore this category of patients is separated in the present material. The presumption that head injury occurs more frequently among children with neurological or severe psychiatric conditions is due to several factors. It may be due to actual conditions, i.e. that head injury is genuinely more frequent than among children suffering from other conditions. It may also be due to the fact that parents of children suffering from neuropsychiatric conditions are more willing to answer questions regarding head injury positively than parents of children with other conditions. It frequently occurs that parents of the former category of children have thought a great deal about the condition and have remembered some injury or other which they consider may explain the condition of the patient. In addition, it is also more probable that the question has been asked previously on other admissions to hospital and they have had the opportunity of thinking about the problem. On the other hand, parents who come with a child suffering from pneumonia, otitis media or other conditions have probably not thought about head injuries and are probably less likely to consider whether such an injury had ever occurred.

In addition to subdivision into neuro-psychiatric and non neuro-psychiatric patients the patients are also divided according to sex in Table 1 It is frequently stated that boys are wilder than girls and that they are more frequently exposed to trauma than girls. Dyggve's figures from a department of child psychiatry show that boys predominate in the ratio 2 1 over girls. Dyggve explains this by the fact that boys are more aggressive than girls and that this necessitates more detailed investigation.

Bohn, similarly reported that post traumatic lesions are more common in boys than girls and relates this to the fact that the behaviour of boys more frequently brings them into traumatic situations In the present material it appears that the incidence in boys and girls is identical. In the group with neurological and psychiatric conditions the incidence for boys was 26 per cent against 31 per cent for girls and in the group with non-neurological conditions 13 per cent of the boys and 12 per cent of the girls had a history of head injury

TABLE 2 *Comparison between percentage of head injuries and age.*

Age in years	Per cent of head injuries Girls	Boys
> 6/12	17	18
> 1	18	18
> 2	19	22
> 3	20	24
> 4	20	25
> 5	21	27
> 6	23	28
> 7	25	29
> 8	26	26
> 9	29	27
> 10	31	27

TABLE 3 *Comparison between the severity of the head injury and the presence of neuropsychiatric symptoms*

Head-injury of	Neuropsychiatric symptoms absent	present
No importance	33 (30 %)	27 (36 %)
Possible importance: single symptoms etc.	44 (40 %)	23 (28 %)
Importance with unconsciousness, etc	33 (30 %)	27 (36 %)

As the possibility of a history of head injury depends upon the age of the patient on admission, Table 2 shows the percentage of the children admitted over a certain age who had sustained head injuries. It will be observed from this that there is no particular difference between boys and girls. The boys possibly sustain their head injuries slightly earlier than the girls but, on the other hand, the incidence stabilizes about the age of 6–7 years while the incidence in girls rises more steadily throughout childhood.

The information collected was further subdivided with respect to the severity of the head injury into three groups. In Group 1 it must be supposed that the trauma has been without any significance. Only an affirmative answer was received and there were no symptoms at all. Among the patients with neuropsychiatric conditions, there were 27 in this group and among the non-neuropsychiatric 33. Group 2 comprises such patients in whom genuine symptoms occurred in the form of vertigo, vomiting, headache and also cases where a doctor was called or the patient taken to a casualty department. In this group the figures are 23 among the neurological patients and 44 in the group of non neurological patients. In Group 3 there was definite loss of consciousness or the patient was admitted to hospital on account of the head injury and, by and large, the injury must be regarded as being of considerable severity. In this group, there were 27 patients from the neurological group and 33 from the non neurological group. The figures in brackets in Table 3 indicate the percentage figure in relation to Groups 1, 2 and 3. It appears from the Table that in approximately one third of the cases in both categories the history of head injury can be ignored and it may be concluded that, in these cases, it has not played any significant role. In a further third the significance of the head injury is questionable and in the remaining third the trauma has probably been of such a nature that it might be responsible for symptoms later on. This corresponds to Otto's statement that approximately one third of the patients, about whom he had information, had been unconscious.

The fact that the distribution is so completely uniform in the groups with and without neuro-psychiatric symptoms may be regarded as indirect evidence that the value of the information obtained from the parents in both groups of patients is the same. This implies that the difference between the 12–13 per cent in the one group and approximately 28 per cent in the other group is a genuine difference and that, regardless of the group, approximately one third of the histories of head injury must be assigned to each of the three degrees of severity.

Summary

In 1100 children consecutively admitted to hospital, inquiries were made con

cerning head injury and information on the subject was obtained in 1134. In the entire material, 16 per cent replied in the affirmative and no difference in the incidence between boys and girls was observed. In patients admitted on account of neurological or severe psychiatric conditions the incidence was significantly greater viz 28 per cent as compared with 1[illegible] per cent in the group of patients without neurological or psychiatric conditions.

References

BORN, S. S.: The reactions of patients to encephalography. *Bull Neurol Inst (New York)* 6 340, 1937.

DYGGVE, H.: Nogle psykologiske synspunkter og erfaringer vedrørende nervøse børn og deres forældre. *Ugeskr Læg* 119 105, 1957.

MELCHIOR, J. C.: To be published.

OTTO, ULF: The postconcussion syndrome in children. *Acta Paedopsychiat* 27 6, 1960.

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Umbilical Vein Pressure in Congestive Heart Failure in the Newborn Infant*

by PAUL M. TAYLOR, JEROME H. WOLFSON, NANCY H. BRIGHT, EDNA L. BIRCHARD and THOMAS J. EGAN

The diagnosis of congestive heart failure in the newborn infant is usually based on clinical findings. Respiratory distress and hepatomegaly are the earliest and most constant signs. Cyanosis, splenomegaly, peripheral edema and rales are noted later in most cases or may not develop at all. Cardiac enlargement is often detected clinically and confirmed by roentgenogram of the chest.

The clinical diagnosis of heart failure may be difficult to make with certainty in some infants with hemolytic disease, the respiratory distress syndrome, septicemia or pneumonia—to mention a few common complications of the newborn period. These conditions may all, of themselves, give rise to combinations of clinical findings that strongly suggest cardiac failure. Heart failure may, on the other hand, complicate the course of these entities. We have found determination of umbilical vein pressure helpful in evaluating infants in whom the diagnosis of cardiac failure is considered. This report presents our experience with such infants.

* Supported by the Twenty Five Club, Womens' Auxiliary of the Elizabeth Steel Magee Hospital.

Patient Material and Method

An attempt was made to measure umbilical vein pressure on all infants delivered at the Elizabeth Steel Magee Hospital over a period of 18 months on whom the diagnosis of cardiac failure was considered. It was not possible to do so in a few cases. Ten infants were studied. Their ages at time of determination of venous pressure ranged from 22 to 76 hrs. Data on infants with severe hemolytic disease are not included in this report.

Umbilical vein pressure was measured in mm of normal saline with the mid-axillary line used as zero position for the manometer. The stump of the umbilical cord was incised and the umbilical vein entered with a polyethylene or polyvinyl catheter which was then advanced from 4 to 7 cm. Readings were accepted when the infants seemed maximally relaxed, and when the fluctuations normally seen with respiration were noted. Repeated measurements were made until consecutive readings agreed within a few mm.

Results

Pertinent information on the infants presented is listed in Table 1. Their umbilical vein pressures are compared with umbilical vein pressures of healthy infants ( ) in Fig. 1. Seven of the 10 infants had

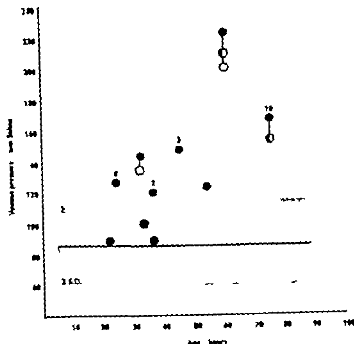


Fig. 1 Umbilical vein pressures of infants on whom the diagnosis of cardiac failure was considered. The heavy horizontal line indicates the mean venous pressure (92 mm saline) of 31 healthy infants 3–60 hours old. The shaded area represents ±2 S.D from the mean venous pressure (2). The numeral above each point refers to the case number noted in Table 1 Half-closed circles and open circles indicate values obtained after removal of a total of 10 ml and 20 ml of blood, respectively

venous pressures greater than 118 mm 2 standard deviations above the mean venous pressure for healthy infants) The incised umbilical vein bled briskly in these infants with elevated venous pressure Six of these 7 infants died, while 2 of the 3 infants with venous pressure within the normal range survived.

Withdrawal of 10 to *0 ml of bl od from the umbilical vein of 3 of the infants in heart failure resulted in an immediate drop in venous pressure (Fig 1) Following venosection, temporary reduction of dyspnoea and of liver size was noted in Case 7 but clinical improvement did not occur in Cases 9 and 10

Umbilical vein catheterization did not produce any discernible adverse effects on the infant In thi series.

Discussion

Elevated umbilical vein pressure indicates failure of the right ventricle with these 3 possible exceptions: 1 during the first hour or so of life following delayed clamping of the umbilical cord ( ) 2. when associated with marked abdominal distention which may of itself increase umbilical vein pressure; and 3 with obstruction of the inferior vena cava above the site of entrance of the hepatic vein and ductus venosus.

The clinical impression of congestive heart failure was confirmed by the finding of elevated venous pressure in 7 of the 8 infants presenting with the triad of dyspnea, tachypnea and hepatomegaly The one subject with these findings and a

TABLE 1 *Information on Infants with Findings Suggestive of Congestive Heart Failure*

Case number	Weight in g	Age onset of distress in hours	Pertinent findings: Tachypnea	Dyspnea	Hepatomegaly	Splenomegaly	Rales	Edema	X-ray	Venous pressure mm saline	Age at venous press. in hours	Age at death in hours	Diagnosis Autopsy findings Remarks
1	4290	1½	+	+	−	−	−	−	C[a]	94	36		Postmaturity Recovered.
2	3870	At birth	+	+	+	−	−	−	N[b]	120	36		Respiratory distress syndrome Recovered.
3	3000	40	+	+	+	−	−	−	C	155	43	50	*Aerobacter aerogenes* septicemia.
4	3390	23	+	+	+	−	+	+	C	100	33		Acute pulmonary edema, Mother diabetic, Recovered.
5	3050	24	+	+	+	+	−	+	C	133	54	55	Hemorrhagic pneumonia, Vascular congestion, Mother diabetic
6	2300	18	+	+	−	−	+	−	ND[c]	94	2	24	Atelectasis and pulmonary hyaline membrane
7	2775	48	+	+	+		+		C	225	60	70	Aortic and mitral atresia, absent left ventricle by hypoplastic left auricle and ascending aorta.
8	3560	At birth	+	+	+	−	−	−	C	125	24	37	Cor triloculare Bonnevie-Ullrich syndrome.
9	3835	8	+	+		−	−	−	C	145	33	46	Bronchopneumonia.
10	3775	72	+	+		−		−	C	173	75	80	Cor triloculare truncus arteriosus, mitral atresia.

[a] Cardiomegaly
[b] Normal cardiac shadow
[c] X-ray not taken.

normal venous pressure (Case 4), the infant of a diabetic mother had a moderately enlarged liver from birth. Two infants in this series had labored and rapid respiration and other findings suggestive of heart failure but normal-sized livers. Both had venous pressures within the normal range.

Walther (3) has reported the case of an infant of a diabetic mother who developed the clinical picture of the neonatal respiratory distress syndrome between the ages of and 3 hrs, and who had an elevated umbilical vein pressure at 3 hrs of age. With drawal of 60 ml of blood lowered the umbilical vein pressure to within the normal range and promptly cured the infant of his respiratory difficulty. Case 4, the infant of a diabetic mother had onset of acute pulmonary edema at 23 hrs. of age his umbilical vein pressure was normal at that time. He improved rapidly after withdrawal of 20 ml of blood, which did not affect his venous pressure and digitalization. This may have been a case of acute left ventricular failure, which is seldom diagnosed with certainty in the newborn infant. These experiences suggest that venesection merits a therapeutic trial in severely distressed infants of

diabetic mothers, no matter what the infant's venous pressure is found to be.

Bonham Carter, Bound & Smellie (1) measured umbilical vein pressures during the first hours of life in a series of infants with the neonatal respiratory syndrome. They found that over half of the infants that survived had elevated venous pressures, while all of the infants who died had venous pressures within the normal range. The explanation of the seemingly beneficial effect of elevated venous pressure in these infants is obscure.

Two full term infants with neonatal respiratory distress are included in the present report. Case 2, with a slightly elevated umbilical vein pressure at the age of 36 hrs, survived, while Case 6, whose venous pressure was normal at 22 hrs. of age, died.

Cardiac failure in the newborn infant was found to be associated with a variety of clinical conditions in this study. The incidence of congenital heart disease as a cause of heart failure in newborn infants is probably higher than the 3/7 noted in this small series. It is important to appreciate that congestive failure may complicate both septicemia and pneumonia in infants. Early diagnosis and treatment of this complication in such cases may be life saving.

When the clinical impression of cardiac failure is confirmed by the finding of elevated umbilical vein pressure, we slowly withdraw sufficient blood (up to 30 ml in mature infants) to effect a definite lowering of venous pressure, and then inject the initial dose of Cedilanid® through the catheter. The ill infant is thus saved the stress of injection and venepuncture.

Summary

Information is presented on 10 infants on whom the diagnosis of cardiac failure was suspected clinically. Measurement of umbilical vein pressure was made in each case, and proved to be of diagnostic value.

References

1. Bonham Carter, R. E., Bound, J. P. and Smellie, Jean M.: Mean venous pressures in the first hours of life. *Lancet* 271: 1220, 1956.
2. Taylor, P. M., Egan, T. J., Birch, Edna L., Bright, Nancy H. and Wolfson, J. H.: Venous hypertension in the newborn infant associated with delayed clamping of the umbilical cord. In preparation.
3. Walther, T.: *Is acute heart failure the cause of the neonatal-syndrome in children born of diabetic mothers? A preliminary report. Ann Paediat (Basel),* 189: 36, 1957.

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Diarrhoea Caused by Deficiency of Sugar Splitting Enzymes I

by H. A. WEIJERS, J. H. VAN DE KAMER, W. K. DICKE and J. IJSSELING

Introduction

In daily practice we are often confronted with diarrhoeas whose cause cannot be found, causal therapy therefore being impossible in these cases. Recently we had to deal with these types of diarrhoea in three children admitted to our clinic.

Patient T., girl, was born on July 15th, 1957 as the first child of healthy parents, delivery was normal, at term. Birth weight 3500 g. After one week of breastfeeding, Lactogen feeding was given, on which the child grew well with normal defaecation. When 5 months old she suddenly fell ill with high fever and diarrhoea. One day later the fever abated, but the diarrhoea persisted in spite of various therapeutic measures (antibiotics, Arobon, buttermilk, protein milk, wheat-free diet etc.). Her increase in weight which had been normal for 5 months, was now arrested.

At admission—at the age of 14 months—the child was dystrophic with a weight of 7900 g and a height of 80 cm; she produced profuse thin, foaming and voluminous diarrhoea that contained much fine mucus. She had a rather bad mood, was petulant and somewhat negativistic. Her muscular development was normal, and physical examination revealed only a somewhat flaccid and swollen abdomen, and rather lively intestinal noises were heard on auscultation.

The following conditions were eliminated as causes of the diarrhoea; enteric infections (amoebic and bacillary dysentery, typhoid and paratyphoid, coli Bray and other pathogenic intestinal flora), worms and giardiasis, coeliac disease, anatomical abnormalities of the gastro-intestinal tract, terminal ileitis, latent iron deficiency, vitamin deficiencies and pancreatic fibrosis, while congenital alkalosis, milk allergy and ganglioneuroma, were discarded as causative factors.

Patient V., boy, was born one month prematurely on October 6th, 1957. Delivery was normal, birth weight was 2300 g. Up to six months he was breastfed with good growth and no diarrhoea. Then persistent diarrhoea (rather thin faeces, yellow, foaming, 2 or 3 times per day).

When, two years old, the boy was admitted to hospital, he was a moderately well, lively, palish child with a somewhat swollen abdomen and slight general hypertonia. During periods of diarrhoea he was unruly and whimpering. On feeding with a protein-milk/water/glucose mixture the diarrhoea disappeared and the boy became cheerful again. Deviations from this diet always caused diarrhoea. Here also all known causes of diarrhoea were ruled out.

His weight on admission was 9800 g (at the age of six months it had been 6500 g) and his height was 77 cm. He was the second child of healthy parents. The first child had died at the age of 15 months from meningitis.

Patient E., boy, was born on August 17th, 1958. Delivery at term was normal and birth weight was 4300 g. He was breastfed for a few days only and diarrhoea commenced

after the first few weeks of life, both on diets of half milk/half water/buttermilk mixtures and on acid/whole milk mixtures; he produced normal stools only on a protein milk/water/glucose mixture. The diarrhoea was foamy and water thin and contained some fine mucus.

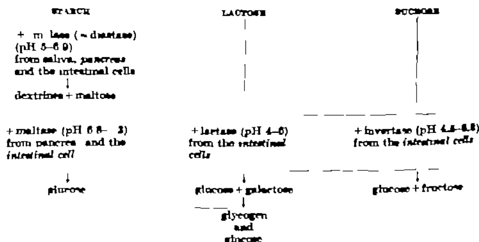
The patient was admitted at the age of one year because of the persistent tendency to develop diarrhoea as soon as he was given a diet other than the protein milk/water/glucose mixture All known causes of diarrhoea were excluded.

On admission he was a pale baby weighing 8700 g and 75 cm in height. Examination did not reveal any abnormal physical signs, apart from a somewhat swollen abdomen. The patient was the fourth child of healthy parents. The eldest child also suffered from diarrhoea.

As the diarrhoea observed in the three patients was thin, foaming and bulky and contained very finely distributed mucus, which is typical for a diarrhoea originating in the small intestine (Goiffon) we had to give serious thought to an absorption disturbance as causative factor Especially in view of the foaming and the low pH of the faeces we thought of fermentation as a result of a disturbance in carbohydrate absorption. The nutritional components to be suspected in this connection are starch, lactose and saccharose, as these substances as such cannot pass the intestinal wall,—in contrast to the monosaccharides glucose galactose and fructose—but are first split in the intestine into monosaccharides: glucose, glucose + galactose and glucose + fructose respectively (see Scheme I) followed by absorption. However if the carbohydrate-splitting enzymes amylase (= diastase) maltase, lactase and invertase (= saccharase) are absent or insufficiently active, starch (dextrins, maltose) lactose and saccharose may reach the ileocaecal region and there be attacked by the intestinal bacteria, to cause a vigorous fermentation with formation of lower organic acids, with harmful results

That one has to reckon with these considerations became clear when Holzel *et al.* published recently about 2 children suffering from chronic diarrhoea caused by lactase deficiency Some cases of starch

Scheme I. *Splitting of carbohydrates for absorption*



intolerance classified by Andersen as coeliac disease are probably also caused by amylase and/or maltase deficiency

Investigations

Carbohydrate tolerance tests and faecal analysis can be used to trace possible deficiencies in carbohydrate-splitting enzymes as causes of diarrhoea.

a. *Carbohydrate tolerance tests*

In a loading test the child—in the fasting condition—is given 2 g of the carbohydrate under test per kg body weight—maximally 50 g—in a 10 % aqueous solution. Then we determined whether the reducing power of the blood increases or not.

Subsequently the enzyme preparation concerned is added to the sugar the hild is given this mixture with cold water and the curve is determined a second time.

The starch loading test is already described by *Althausen* analogous to the casein loading test.

T test the lactase activity in the intestine, the lactose curve was first applied by *Holzel* and co-workers.

If the curve only rises with the addition of an enzyme preparation serious thought should be given to an enzyme deficiency

When interpreting curves, one should realize that their course depends not only on the rate of supply f monosaccharides to the blood, but also on the rate of removal of these substances from the blood. Supply and removal are, in turn, resultants of va ious factors (Scheme II).

It was our experience in several children that a flat loading curve was caused by a marked carbohydrate hunger of the liver This phenomenon could be corrected by giving the patient an extra amount of glucose during 3 days.

Some cautiousness is therefore warranted in the interpretation of the various curves. Usually however they can be evaluated as follows (Schemes III and IV).

The situation is normal if, after loading with 2 g *lactose, maltose or saccharose* per kg body weight, an increase of the reducing power of the blood serum is found of approximately 50 mg %, expressed as glucose This means that both the enzyme activity and the absorption are good.

If, however the curve rises less than 20 mg %, we should expect a too low lactase maltase or invertase activity or a disturbance in the absorption. To find out which is responsible the curves are repeated but now with addition of the enzyme concerned. If the curve rises after this addition, the assumption of too low enzyme activity in the intestine, is justified. If however the curve remains flat in this case also, the loading should be repeated once more namely with the monosaccharides which form the elements f lactose, maltose and saccharose;

SCHEME II *Factors nfluencing the course of a carbohydrate loading curve*

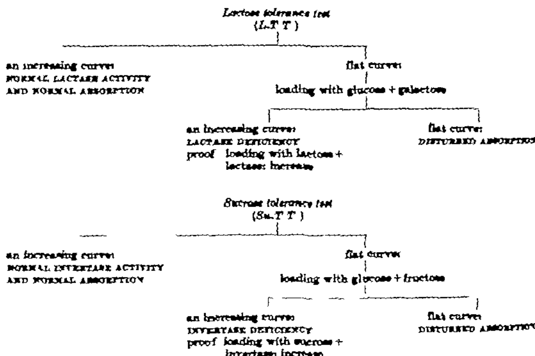
SUPPLY

- dosage
- b speed of absorption
  - 1 speed of gastric emptying
  - 2 fermentative splitting in the intestine
  - 3 absorption by the cells of the intestinal wall and transport through the portal vein
- (c) glycogenolysis

REMOVAL

- (a) glycogen synthesis
- b direct combustion in the tissues
- threshold value of the kidney

Scheme III



Scheme IV

Starch tolerance test
(*St.T.T.*)

an increasing curve:
ORM MYL SE AND MALT SE
CTIVITY AND NO MAL BSORPTION

a flat curve:
A. DISTURBED ABSORPTION
proof: loading with glucose: no increase or/and B. MAL
TASE and/or MYLASE DEFICIENCY

followed by

Maltase tolerance test
(*M.T.T.*)

increasing curve:
AMYL E DEFICIENCY
proof: loading with starch am lase: increase

flat curve:
A. MALTASE EFI ENCY
proof: loading with starch malt se: increase
B. MALT SE AND AMYLASE EFICIEN Y
proof: 1. loading with starch maltase: no increase
2. loading w th starch malt se am lase: increase

in other words, with a mixture of equal amounts of glucose and galactose and glucose, and glucose and fructose, respectively In this case a flat curve points to a disturbance in the absorption mechanism.

As the breakdown of starch to the monosaccharide glucose takes place in two stages, for which two enzymes are required, i.e. amylase and maltase a loading with starch —2 g per kg bodyweight, with a maximum of 50 g—which results in an increase of the reducing power of the blood serum of about 30 mg %, expressed as glucose, points to a good amylase and maltase activity as well as to a normal absorption.

If the curve of the starch tolerance test (St T T) does not rise, however we should again differentiate which of the three factors mentioned just now is responsible

The absorption is to be judged by means of a simple glucose load; a flat curve points to a disturbance in the absorption.

If the glucose tolerance test (G T T) shows a normal rise, however the loading should be done with maltose as a flat maltose tolerance test (M.T T) in this case indicates a maltase deficiency while in the case of a normally rising M.T T the maltase activity is good. A deficient maltase activity can even be demonstrated in more detail by repeating the M.T T once more but now under the addition of maltase. Now the curve should show normal rise A maltase depletion may cause a flat St T T curve, which can be investigated by loading with starch under addition of maltase. The curve should then rise

Besides a maltase deficiency there may also be an amylase depletion. In this case loading with starch to which maltase has been added will not cause a rise while addition of maltase and amylase will do so

If both G T T and M.T T show a normal rise, the flat St.T T can only be caused by an amylase deficiency This is to be proved by once again loading with starch, but in this case only with addition of amylase the curve showed then rise.

b *Examination of the faeces*

Based on a diet that only contains glucose as a carbohydrate—and after previous demonstration that the glucose absorption is good—the harmful activity of the carbohydrates starch, maltose, lactose or saccharose can be tested clinically using the occurrence of diarrhoea as criterion, by replacing the glucose by an equivalent amount of one of these carbohydrates.

Moreover chemical examination of the faeces may afford an understanding of the severity and nature of the disturbance (chemical methods, see Addendum)

Bacteriological examination can be carried out by using a direct quantitative typing of the more significant components of the faecal microflora as diagnostic criterion. For this purpose the viable numbers of the fol

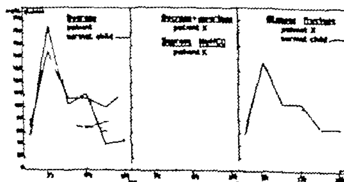


Fig 1 Sucrose tolerance test in invertase deficient patient X

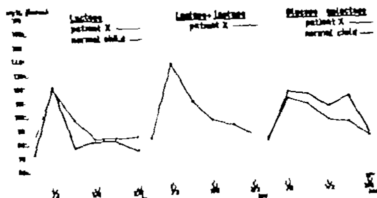


Fig. 2. Lactose tolerance test in non lactase deficient patient X.

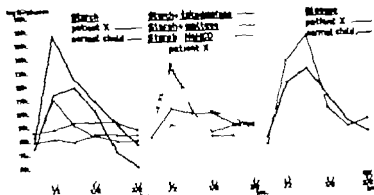


Fig. 3. Starch tolerance test in non amylase deficient patient X.

lowing organisms were determined in fresh stool samples both during episodes of diarrhoea and while recovering:

(i) three broad general classes of bacteria;
(ii) four typical groups of faecal bacteria,
(iii) yeasts

Results of the examinations

a. *Carbohydrate tolerance curves*

Patient X After *saccharose* loading there was a flat curve which points to an *invertase deficiency* an absorption disturbance being excluded because loading with glucose + fructose caused a normal rise Some rise was indeed observed if the loading was done with saccharose to which invertase had been added (Fig 1)

After loading with *lactose*, a normally rising curve was determined, which means that a lactase deficiency was to be ruled out (Fig 2)

A flat curve was found after loading with *starch* (Fig 3) as also after loading with *maltose* (Fig 4) at any rate in January In September however some rise was observed. This was not caused by a disturbed absorption, as the glucose tolerance curve showed a normal rise (Fig 3) Neither was it possible to explain the flat course of the starch loading curve by

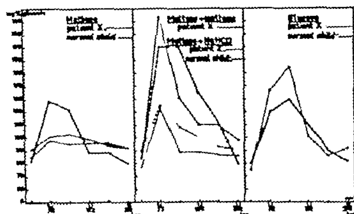


Fig. 4 Maltose tolerance test in maltase deficient patient X.

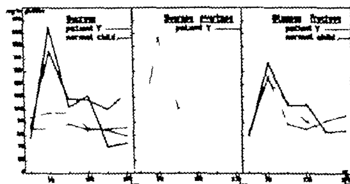


Fig. 5 Sucrose tolerance test in invertase deficient patient Y

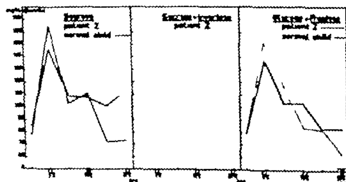


Fig. 6 Sucrose tolerance test in invertase deficient patient Z.

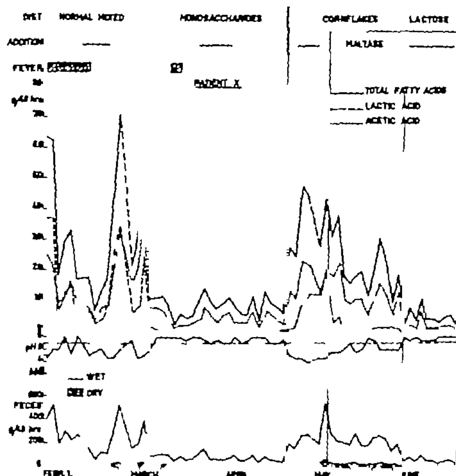


Fig. 7. Faeces examination in patient X, suffering from maltase and invertase deficiency on basic diet supplemented successively with different carbohydrates without and with enzymes added from February till June.

assuming an amylase deficiency as a normal quantity of amylase was determined by means of duodenal sounding. Moreover the starch tolerance curve was also flat with addition of takadiastase (Fig. 3). The explanation should therefore be sought for in a *maltase deficiency*. Loading with maltose to which maltase had been added, indeed resulted in a normally rising curve while a normal rise was also obtained by loading with starch to which maltase had been added (Fig. 4).

Patients Y and Z. A flat saccharose tolerance curve was also determined in these children, the curve did rise if invertase was added during the loading (Figs. 5 and 6). As the rise of the two glucose fructose curves was normal the conclusion was *invertase deficiency*.

In none of the two patients could a lactase, maltase or amylase deficiency be demonstrated.

b. *Analysis of the faeces*

In patient X, the various diet periods showed the following trend as regards the

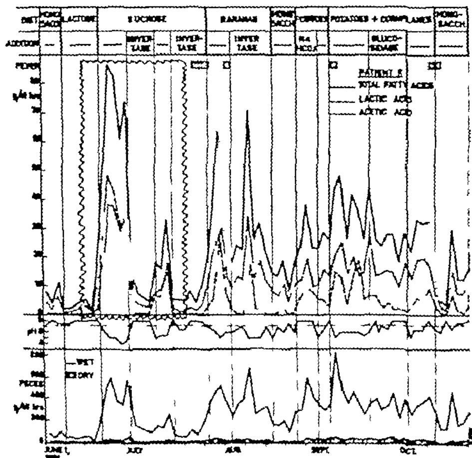


Fig. 8. Continuation of Fig. 7 from June till October; the encircled part is given more detailed in Fig. 10

quantities of faeces and its composition (Figs. 7 and 8).

As shown in the above figure the child suffered seriously from diarrhoea when being on a normal mixed diet. She produced up to 500 g faeces per two days while she also excreted a considerable proportion of lower organic acids, mainly acetic acid and lactic acid, increasing up to 7 g per 48 hours: these acids are a criterion for the activity of the fermentation flora in the intestine which, in this case, was abnormally great. It is therefore no cause for wonder that the pH of the faeces was low, even as low as pH 4.5. The fat excretion per 48 hours was only slightly above normal.

The patient was subsequently given a diet that contained glucose as the only carbohydrate. This diet was composed as follows:

.30 a.m. 150 g curds + 25 g Nutroma + 35 g glucose

9.30 a.m. the press juice of one orange

11.30 a.m.	100 g green vegetables + 30 g lean meat + one spoonful of gravy
2.30 p.m.	like 7.30 a.m.
5.30 p.m.	like 7.30 a.m.

This diet led surprisingly rapidly to a markedly favourable effect. The volume of the stools decreased to less than 100 g per 48 hours, the acid excretion fell from 7 g to 1 g and the pH rose to 8. The fat excretion, too decreased to entirely normal values.

The improvement was also striking in clinical respect. Her negativistic, slightly irritated adjustment to her surroundings disappeared and she became a cheerful, active little girl.

The glucose-containing diet was continued for 1½ months, during which period the girl remained in an excellent condition without producing diarrhoea.

Then the diet was changed only in this respect that a part of the glucose in the diet was replaced by a calorically equivalent quantity of starch in the form of ground cornflakes, a product consisting largely of starch and which can easily be used in the diet instead of glucose, by mixing it with the curds. The patient reacted promptly to this change with a considerable rise of the amount of lower organic acids and a corresponding fall of the faecal pH, shortly afterwards followed by diarrhoea.

In view of the result of the duodenal intubation and the flatness of the curve after loading with starch to which takadiastase had been added, the expectation was justified that the diarrhoea was not caused by an amylase deficiency but rather by a shortage of maltase and a maltase-containing preparation was therefore added to the diet in a dosage of 18 g three times per day given after one third of the meal had been eaten. A favourable influence was observed, the diarrhoea decreased, while the excretion of lower organic acids also became somewhat less especially when the quantity of maltase preparation was raised up to 27 g three times daily.

Although an improvement was perceptible it was not until reverting to the glucose diet as used in the previous period, that it became evident that this improvement had been only partial, as the glucose diet was directly followed by a further favourable reaction to such a degree that the diarrhoea disappeared entirely as also the increased acid excretion and the faecal pH rose. In our opinion, the cause of the moderate change for the better after administration of maltase, finds an explanation in the fact that maltase is very acid sensitive resulting in a considerable loss of activity during the passage through the stomach.

When, subsequently 3 × 12 g of the glucose of the diet was replaced by 3 × 12 g lactose this had no harmful sequelae. This confirmed the expectation that there was no lactase deficiency—an expectation formed already on the basis of the lactose loading curve.

Finally 3 × 12 g glucose of the glucose-diet was replaced by an equal amount of saccharose. In view of the saccharose loading curve diarrhoea was again to be expected, it did in fact occur immediately and very violently accompanied by a considerable excretion of lower organic acids, the pH of the faeces falling as low as 4.

The favourable reaction after addition

of 4 × 250 mg of an invertase preparation occurred as promptly as the unfavourable effect of saccharose without invertase

The experiment was repeated once more in general with the same result It was this time shown that even half the amount of invertase had a favourable effect.

Now overripe bananas were added to the diet (bananas contain saccharose and little or no starch, especially when over ripe) to which the patient reacted immediately and violently with diarrhoea and markedly increased acid excretion.

Addition of invertase had the expected favourable effect In the beginning the diarrhoea returned, however but disappeared again although no special measures were taken. Some days later it transpired that the unexpected aggravation of the diarrhoea and of the acid excretion had been caused by the eating of sweets containing several grammes of sucrose which the patient had been given by her grandmother in spite of our prohibition.

As a deficient enzyme activity might not only be caused by an enzyme shortage but also by too low a pH in the duodenum, starch was subsequently given—as potatoes—with addition of 3 × 750 mg NaHCO per day This addition had, however no favourable influence

We also studied whether the enzyme glucamylase exerted a favourable influence on the digestion of starch as this enzyme is less susceptible to the influence of gastric acid than maltose However the breakdown of starch by glucamylase differs from that by amylase + maltase as glucamylase at once splits off glucose without the formation of maltose as an intermediate product. It is therefore capable of compensating a maltase shortage.

As, however the pancreas activity of the patient was normal, a competition between the amylase and glucamylase activities was to be expected. In other words, there was a fair chance that in spite of the presence of glucamylase maltose would still be formed as glucamylase is able to break down starch directly to glucose but leaves maltose intact.

Glucamylase had indeed no favourable effect; the diarrhoea and the high acid excretion persisted, on the other hand, the lactic acid excretion decreased under the influence of glucamylase.

The original glucose diet was finally given again following which the diarrhoea disappeared.

As we therefore did not succeed in remedying the deficient maltase activity by some preparation or other the child was discharged on the glucose diet This may be supplemented with saccharose provided invertase is also given. The child (Patient X) is in very good condition on this diet and no longer suffers from diarrhoea.

Patients Y and Z (Fig 9) showed the same rapid intense reaction to saccharose as patient X. They also showed a prompt recovery after addition of invertase to the diet.

On *differentiation of the lower organic acids* in the diarrhoea periods (see Fig 10) it appears that the faecal fatty acids consist of more than 90 % of lactic acid and acetic acid. A particularly remarkable feature is the high excretion of lactic acid, as this acid was almost entirely absent in all three patients in periods

TABLE 1 *Bacteriological differentiation of faeces as influenced by diet in a patient suffering from maltase and invertase deficiency (Mossel)* [1]

Bacterial counts per one gramme of fresh faeces	Diet with maltose or sucrose	Same plus corresp. enzymes; or diet with glucose only
Total aerobic count	$0.2 \quad 10^{10} - 0.4 \times 10^{11}$	$0.1 \quad 10^{10} - 0.8 \quad 10^{10}$
Total anaerobic count	$0.7 \quad 10^{9} - 0.4 \quad 10^{11}$	$0.1 \quad 10^{10} - 0.2 \quad 10^{10}$
Aerobic proteolytic count	$0.1 \quad 10^{3} - 0.2 \quad 10^{6}$	$0.9 \times 10^{4} - 0.5 \quad 10^{5}$
Enterobacteriaceae	$0.3 \quad 10^{9} - 0.8 \times 10^{9}$	$0.8 \times 10^{6} - 0.3 \quad 10^{10}$
Faecal streptococci	$0.4 \quad 10^{9} - 0.4 \quad 10^{1}$	$0.9 \times 10^{7} - 0.7 \times 10^{8}$
Sulphite reducing clostridia	$0.4 \quad 10^{6} - 0.6 \times 10^{7}$	$0.1 \quad 10^{4} - 0.6 \quad 10^{6}$
B. lactobact. bifidum	$0.2 \times 10^{9} - 0.8 \times 10^{11}$	$0.3 \quad 10^{8} - 0.8 \quad 10^{10}$
Yeasts	$0.6 \quad 10^{4} - 0.7 \quad 10^{7}$	$0.4 \quad 10^{4} - 0.1 \quad 10^{7}$
pH value of faeces	4.7 – 5.8	6.4 – 7.2

rides, while the other two little patients though capable of converting starch into glucose were just as patient X incapable of splitting saccharose into glucose + fructose. Thus the non-absorbed disaccharides and starch are attacked by the bacteria further down the intestine which results in fermentation with subsequent formation of organic acids, mainly acetic and lactic acid. These acids, and possibly also other metabolites of the bacterial flora irritate the intestine which reacts to this with increased peristalsis excretion of fluid and mucus formation due to which the absorption is disturbed with subsequent diarrhoea.

Of the patients discussed one therefore suffered from a *maltase* + *invertase* deficiency the other two only from an *invertase* deficiency.

The invertase deficiency was excellently compensated for by an invertase preparation, and was, of course also remedied by a saccharosefree diet.

We failed to compensate the maltase deficiency with the help of enzyme preparations, as the maltase and glucosidase preparations used were ineffective in this respect. It may be that a combination of these two preparations, provided with a coating so that the maltase is not inactivated in the stomach, will lead to favourable results. This is under investigation at present.

Sodium bicarbonate was useless for protecting the maltase or exerting a favourable influence on its activity.

Recently we were also able to confirm in a three-months old infant with a stubborn diarrhoea the observation made by Holzel that a diarrhoea can be caused also by a lactase deficiency whereas no sugar was excreted with the urine. The diarrhoea was stopped and the baby started growing by excluding only lactose from the diet whereas adding lactose to the diet caused again diarrhoea. The child showed also a flat lactose tolerance curve.

So far we cannot state with certainty whether these enzyme deficiences are of a familial nature and permanent or only transient.

Holzel and co-workers demonstrated

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[1] The authors are greatly indebted to Dr D. A. A. Mossel for the bacteriological investigations and for the interpretation of the results.

lactase deficiency in two children out of one family the eldest of whom was already 10 years of age and this makes one inclined to believe that these enzyme deficiencies are familial rather than transient and this belief is strengthened by a recent observation we have made on the 13-year old sister of patient Z, in whom we find an invertase deficiency which had not led so far to trouble clinically as she consumes practically no sugar

It may be concluded that deficiencies of carbohydrate splitting enzymes in the intestine may give rise to chronic diarrhoea with all the consequences involved, even with arrest of growth. This should always be kept in mind when one tries to find the cause of an intractible diarrhoea.

Summary

In 3 children suffering from chronic diarrhoea in which all normally occurring causes (including coeliac disease) were ruled out the absence (or defective functioning) of invertase and/or maltase was proved to be the cause The diarrhoea was arrested with a monosaccharide diet, and in the case of invertase deficiency also an invertase preparation helped. The observation of Holzel *et al.* that a chronic diarrhoea—without excretion of lactose with the urine—also can be caused by lactase deficiency could be confirmed in another child.

The criteria were the severity of the diarrhoea, the pH of the faeces and the excretion in the stools of lactic acid and the volatile lower fatty acids gas-chromatographically differentiated into formic acid aceti acid, propionic acid, (iso)-butyric acid, (iso)valeric acid and caproic acid

It appeared that for the diagnosis of diarrhoeas caused by carbohydrate intolerance the lactic acid content of the faeces is especially valuable Differentiation of the faecal flora gives no help.

In no case more than traces of sugar where excreted with the urine.

Acknowledgement

W are greatly indebted to *Dr Loggers* and *Dr Vegels* of the Nederlandsche Gist- en Spiritusfabriek of Delft, for their kindness to put the enzyme preparations at our disposal and for the interest shown in our work.

Addendum

After delivery of this manuscript we could observe two resp. three patients in two different families suffering from lactase deficiency giving support to the presumption that these deficiencies are familiar

Experimental part

Faeces investigation

1 Quantity
2 Dry substance
3. pH colorimetrically
4. Fat content according t *Van de Kamer et al.*
5. Content of lower molecular organic fatty acids according to *Gerritsma*

A maximum of 10 g well-mixed faeces in 50–75 ml water are transferred to a calibrated flask of 100 ml, after good trituration After addition of 3 ml $FeCl_3$ solution the mixture is made up t 100 ml, following which 3 g $Ca(OH)_2$ is added. After vigorous shaking, the flask is left standing for 5 minutes subsequently the mixture is filtered off—under suction—over Büchner f lter (9 cm) provided with three discs of filter paper (Schleicher and Schüll No. 595) (*Geiffen*).

Prepared as follows: solution of 10 g $FeCl_3 \cdot 6H_2O$ in water made up to 100 ml, under addition of 2 drops of concentrated HCl.

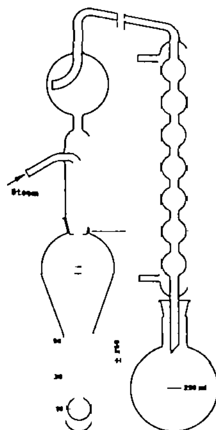


Fig. 11 Destillation apparatus according to Friedemann.

In this way the higher fatty acids are separated from the faeces in the form of Ca soaps, while the lower f tty acids are collected] uantitati ely in the filtrate.

a. *Determination [lactic acid*

In a part f the filtrate, containing about 1 mg f lactic acid, if need be made up to 5 ml, the lactic acid is determined according t the method of *Long* with the f l lowing mo lifications [oposed by *Gerr t ma* Acet ldehyde is transferred to the bisulphit solut on with trogen instea l f a r crystal line potassi m metabisulphit (K_2SO_3 SO_2) is used instead f the] l erized s l m metabi lphit wl le the bead sed to wash the strea i w tl bisulphit should not have a lameter larg r than 3 m

b *Determ nation of the total content of lower volatile fatty acid*

50 ml of the calcium filtrat of the faeces are distilled in a distillation apparatus according to *Friedemann* (see Fig. 11)

After addition f 8 g $MgSO_4$ and 4 ml 10 N-H_2SO_4 steam is bubbled through, while the flask i heated, in the beginning with a big flame until the volume has been reduced to 30 ml.

Then the flame is adjusted in such way that the volume remains about 30 ml, and 250 ml are distilled over in 45 minutes. After bubbling through of nitrogen, titration is done with 0 1 N N OH on phenolphthalein (t_1).

Then the content of lower *volatile fatty* acids per 100 g faeces is:

$$2 \quad 10 \times 0\ 1 \quad t_1 \text{ mEq}$$

The content in mg of each acid separately is calculated from the titrations after gas-chromatographic separation (see sub c).

If much lactic acid is also present, a correction can be made for this, as, according to *Friedemann* 3 % of it is carried over in the dist llation.

c. *Determination of the content of each of the lower fatty acids separately*

After addition f a few drops of 0.1 N NaOH the neutralised distillat obtained nder b is evaporated into a small olume. This is washed with water into an Erlenmeyer flask of 50 ml and dried by evaporation on a steam bath. Then are added 4 ml ether and 0.–5 ml 4 N sodiumhydrog lphate If there is more than 0 9 mEq total acid present, more sodi umhydrog lphat solution should be added namely such quantity that there is an excess f abo t 10 %. The mixt re is vigorously stirred and the salt resid e thoroughly loosened with a stirring rod Subsequently 0.5 g dry N $_2SO$ is ad led for each 0.–5 ml sodiumhy lrosulphat sol ition, and the sol tion shaken unt l it has become lear After 30 minutes the sol t is filtered through a small wad of cot t n wool int a cal l t l flask of 10 or –3 l. The resid s l ted with [tions

of 1 ml ether. After mixing with ether and making up to 10 or 25 ml, formic acid, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid and caproic acid are quantitatively determined by gas-chromatography according to *James & Mart* as modified by *Van de Kamer, Gerritsma & Wansink*.

References

1. ALTHAUSEN, T. L. and UYEYAMA, K.: A new test of pancreatic function based on starch intolerance. *Ann Intern Med*, *41* 563–575, 1954.
2. ANDERSEN, D. H.: Celiac syndrome. VI. The relationship of celiac disease, starch intolerance and steatorrhea. *J Pediat*, *30* 564–582, 1947.
3. DAHLQVIST, A., MOSSBERG, O. and SØNDERGAARD, G.: Lactosuria and amino-aciduria in infancy. *Acta Paediat*, *49* 281–290, 1960.
4. DURAND, P.: Lattosuria idiopatica in una paziente con diarrea cronica ed acidosi. *Minerva Pediat*, *10* 706, 1958.
5. DURAND, P.: Lactosurie et saccharosurie. *Probl Act Paediat*, *4* 496, 1959.
6. DURAND, P.: Insuffisante hydrolyse intestinale du lactose. *Pédiatrie*, *15* 407, 1960.
7. FRIEDEMANN, T. E.: The identification and quantitative determination of volatile alcohols and acids. *J Biol Chem*, *123* 161–184, 1938.
8. GERRITSMA, K. W.: Quantitative determination of the lower fatty acids in faeces by partition chromatography. Thesis Utrecht, 1954.
9. GOIFFON, R.: Manuel de coprologie clinique. 5ème éd. Paris, Masson et Cie, 1949.
10. HOLZEL, A., SCHWARZ, V. and SUTCLIFFE, K. W.: Defective lactose absorption causing malnutrition in infancy. *Lancet*, *1* 1126–1128, 1959.
11. JAMES, A. T. and MARTIN, A. J. P.: Gas-liquid partition chromatography: the separation and micro-estimation of volatile fatty acids from formic acid to dodecanoic acid. *Biochem J*, *50* 679–690, 1952.
12. JEUNE, M., CHARRAT, A., COTTE, J., FOURNIER, P. et HERMIER, M.: Sur un cas de lactosurie congénitale. *Pédiatrie*, *15* 411–419, 1960.
13. KAMER, J. H. VAN DE, GERRITSMA, K. W. and WANSINK, E. J.: Gas-liquid partition chromatography: the separation and micro-estimation of volatile fatty acids from formic acid to dodecanoic acid. *Biochem J*, *61* 174–176, 1955.
14. LONG, C.: The stabilization and estimation of lactic acid in blood samples. *Biochem J*, *40* 27–33, 1946.
15. MOSSEL, D. A. A.: The suitability of some obligate anaerobic nonsporeforming enteric bacteria as part of a more extended bacterial association as indicator of faecal contamination of foods. *J Appl Bacteriol*, *21* 184–192, 19..
16. MOSSEL, D. A. A. and KRUGERS DAGNEAUX, E. L.: Bacteriological requirements for and bacteriological analyses of precooked ('instant') cereals and similar foods. *Antonie van Leeuwenhoek*, *25* 230–236, 1959.

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CASE REPORTS

Annular Pancreas

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The occurrence of aberrant pancreatic tissue in various abdominal organs, especially in the gastro-intestinal tract, is not infrequent. The aberrant tissue is usually small, does not give rise to symptoms and is commonly found incidently at operations and autopsies. In some cases, however it can actually give rise to obstruction, invagination (16) or other disturbances.

In addition to mechanical complications pancreatitis (5 18) malignant degeneration and, occasionally hypertrophy of the islands of Langerhans with resulting hyperinsulinism (3) occur in the aberrant tissue

Danzis has classified ectopic pancreatic tissue as I—aberrant pancreatic nodules having no connection with the pancreas proper II—annular pancreas occurring less frequently than the nodular forms.

A combination of these types has only been reported sporadically (6)

This article is chiefly concerned with annular pancreas with reference to two infants observed in the Pædiatric Clinic of the University of Amsterdam

Case 1

The patient, a male infant, the first child of young healthy parents (negative family history) was admitted to the Clinic on August 22, 1953, a few days after birth.

The birth weight was ..700 g. When the infant received its first breast feeding, it was observed that the child did not drink well and vomited (non projectile) a great deal, this condition remained unaltered in the course of the following days. The vomitus was egg yellow; there was no admixture of blood. On the third day meconium was passed At the time of admission the child was sluggish extremely hypotonic; the skin turgor was markedly decreased. The large fontanel was not sunken. There were mongoloid stigmata but no abnormalities were found in heart or lungs; liver and spleen were not palpable There was, however clearly visible peristalsis in the epigastric region.

Shortly after the first feeding the infant vomited, in a large wave canary yellow fluid (acid reaction) Despite the administration of a hypodermoclysis and the discontinuation of oral feeding, the patient vomited a few more times.

On Aug *6, roentgenological examination revealed a large gas bubble and a few smaller ones in the upper abdominal quadrant Lipiodol introduced into the stomach. as



Fig 1.

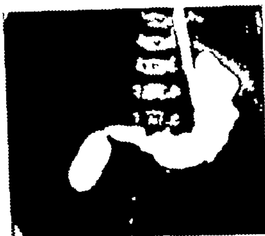


Fig 2.

Fig. 1. Survey film of the abdomen with the presence of air in the stomach and duodenal bulb only

Fig. _ Lipiodol, introduced into the stomach, passed the pylorus, but was retained in the descending segment of the duodenum.

able to pass the pylorus but was retained in the last portion of the descending segment of the duodenum (Fig 1). After four hours, all contrast media had collected in this area.

Due to the child's poor general condition operation was postponed till the next day

At operation, it appeared that the stomach and the foremost ⅔ portion of the duodenum were markedly distended, the lumen of this latter organ showed a diameter of some four centimeters. At the border of the distended and non-distended segment, the intestine was found to be affixed to the posterior abdominal wall as well as t other intestinal loops, which accounted for the stenosis.

Directly caudal to the stricture the in testine was completely collapsed.

Clea age of the adhesions enabled mobilization f the duodenum and it was then simple matter to press the gaseous con tents of the stomach through the stenosis. After the operation the child still vomited bile but the abdomen was no longer distended, and peristalsis could be clearly heard.

On September 4, signs of bdominal distension appeared again. Roentgenologic ex amination demonstrated the same condi tion as had been present prior to the operation. In a second operation a isoperistaltic duodeno-jejunostomy was performed. The post-operative course was good initially but was complicated, thereafter by dyspeptic symptoms and a fever of up to 39 4 C. The general condition deteriorated, and despite withholding of oral feeding and administration of plasma- and glucose-salt infusions, the patient expired.

Post-mortem examination revealed

No free fluid in the abdominal cavity

The anastomosis between the jejunum and duodenum to be adequate.

The proximal portion of the duodenum was extremely distended, and a f brous ring was found approximately 3½ cm from the pylorus. This ring, ½ cm thick with width of a few mm caused constriction of the duodenum leaving only a narrow opening.

Microscopic examination disclosed the presence of pancreatic tissue containing many granulocytic inflammatory inf ltrations.

Case

The patient, female infa t born on November 9, 1938, was the first ‹ ld of elderly primiparous mother, both parents

were healty. The gestation period was uneventful, with the exception of a toxicosis and accompanying pre-eclamptic signs at the close of the pregnancy. In the thirty-seventh week the membranes broke spontaneously and approximately eight liters of amniotic fluid were lost. A spontaneous birth followed, the child cried immediately. The birth weight was 2500 g.

On the first day of birth, it was observed that fluid ran from her mouth; there were no accompanying attacks of cyanosis. The following day the child vomited everything (non projectile). The vomitus was not bilious, there was no admixture of blood. Meconium was passed shortly after birth. The infant showed a continual loss of weight and became increasingly apathetic.

At admission, on December 3rd, a deathly ill, severely dehydrated, and extremely apathetic child was seen. The skin was gray cyanotic in colour and showed practically no tonicity. There were no signs of neck stiffness; heart and lungs were normal. The bdomen was markedly sunken, there was no visible peristalsis, and palpation did not reveal any enlargement f the liver or spleen, or any abnormal resistances. Introduction of probe into the stomach ruled out the possibility of an atresia f the oesophagus. ral feeding was a bst tuted by intravenous administration f plasma and glucose-salt solutions.

A great mprovement in the general condition could be observed on the follow ng day but the infant remained, more or less, apathetic. The single om t s that lay contained haemat n and showed an ac l reaction; a small mount f fresh blood w s also found in the stools. In estigation f the coagulation t me revealed the follow ng deviations the prothrombin t me (Q ick) showed a m h great r let l nce that ... usually found ... fifty-one seconds, ... fourteen second. The plasma ... globul concentrat ... (normal 11 16 U) proth ... (240–300 U) ... (normal 70–120). Intramusc ... nistration of 1 mg Konakion (vitamin K of Hoffmann La Roche) brought the prothrombin time back to a normal value.

On December 7 the child was fed very cautiously but due to a recurrence of vomiting, this procedure was discontinued.

December 8 another attempt at feeding was initially successful, but later on a small amount of fluid ran from the patient's mouth.

Roentgenologic examination: a survey film of the abdomen revealed the presence of air in the stomach and duodenal bulb, but there was a total absence of gas in the remainder of the abdomen. According to Clatworthy & Lloyd, the "U"-shaped form taken on by the gas in the stomach and duodenum, is typical for an obstruction of the duodenum (Fig. 2).

Operation: The transverse colon and mesocolon were adherent to the lower edge of stomach and duodenum; the intestines were in a generally collapsed condition; the colon was extremely thin.

Careful inspection f the duodenum revealed that its descending portion, lying deep in the posterior abdominal ca ty as encircled by a band of pancreatic tissue ½ cm in width. Proximal to this band, the duodenum showed a marked distension. An isoperistaltic, side-to-side duodeno-jejunostomy w s performed. The postoperative course was complicated by bilious vomiting; however a postoperat ve survey film of the bdomen demonstrated gas-filled intestinal loops.

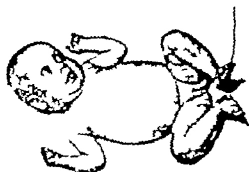


Fig. 3. P t ... ge of ... months.

The patient's general condition remained satisfactory on intravenous administration of plasma-glucose-salt solution and after 16 days the child adapted itself quite well to oral feeding.

On February 14, 1950, the child was discharged from the clinic in good condition, weighing 3500 g.

Follow-up examination on May 23, 1950 disclosed that the child no longer vomited, at well and had gained 2410 g (Fig. 3).

On April 7 1960 we saw the child again; it was in perfect condition.

Occurrence and clinical picture

Annular pancreas is a developmental anomaly in which the descending portion of the duodenum becomes encircled by a ring of pancreatic tissue.

This disturbance was first described by Tiedemann (1818) in an adult man and woman. Ecker (186_) introduced the term annular pancreas and Vidal (1905) was the first to describe this condition in an infant the patient being three days old. Since that time many cases have been published (9 13 14 20 1 23 24 25). The majority of the cases hav been reported in males. In 1953 Swynnerton & T rner collected sixty four cases fifty of whom were males. Many cases did not show any associated clinical signs and have only been reported in the literature as incidental findings after the death of the individual from other causes.

Of fifty-eight cases reported by Beckford & Williamson in 1954 only eighteen showed clinical signs.

The first symptoms usually are the result of an acute or recurrent closure of the duodenum and can take the form of partial or total obstruction (the two presented here). In the latter and tion, symptoms will become manifest shortly after birth a partial closure may become complete at a later age (Custer & Waugh. 74 yr) e.g oedema of the mucosa. The presence of *bile pigments in the vomitus* is dependent on the occurrence of the pancreatic ring proximal or distal to Vater's papilla.

Roentgenological examination with a contrast medium is generally unnecessary in a neonate with total occulsion. The presence of gas in the stomach and its absence in the abdomen, visualized on a plain survey film usually gives substantial evidence for a diagnosis. The use of a contrast medium is however indispensable for the investigation of the partial closures, as manifested in older patients.

As was seen in the first patient described, an acute or chronic pancreatitis can appear accompanied by severe abdominal pain and distension a rapid pulse and a rise in temperature. The inflammation can be localized in the normally present pancreas, in the aberrant pancreatic tissue ring or in both.

In a few cases pressure on the bile ducts has resulted in the development of jaundice (1 22). Frequently the condition is accompanied by a gastric or duodenal ulcer which may give rise to haematemesis and/or melaena. The mechanism as regard this latter situation is not apparent some investigations assume that it is due to a tasis of gastric juices. Cohen described a case where the protective mucous membrane in the pyloric area had been ulcerated he ascribed this t an irri t tion by the pancreatic juices others claim that the ulcer is due t pressure trophy. Whether an ulcer had develop l

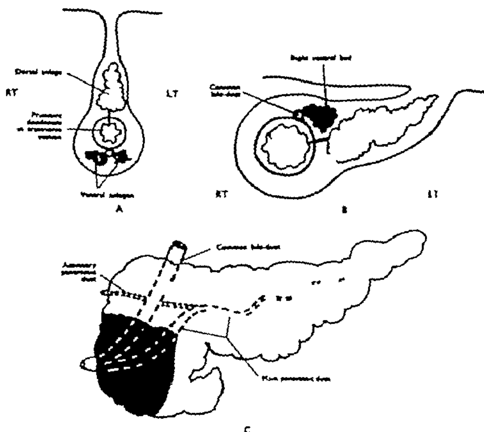


Fig. 4 Normal pancreatic development. A. Initial stage with dorsal and ventral anlage. The left ventral anlage (shaded) trophies and disappears. B Later stage with rotation of the duodenum. C. Adult form. (After Anderson & W pshaw)

in our second patient (haematin containing vomitus) cannot be ruled out with any certainty but we are inclined to believe that this was not the case It is most probable that the haematemesis was due to the very low concentrations of proconvertin and prothrombin. Owing to the fact that these coagulation factors were normalized after the administration of 1 mg vitamin K, it can be concluded that the deficiency of these factors was not a result of hepatic insufficiency but rather due to a lack of vitamin K. This can be further accounted for when we consider that the bacteria necessary for the production of vitamin K in the intestines were not able to reach the bowels, resulting in a disturbance in vitamin K production.

Duodenal occlusion, either due to an atresia or to annular pancreas, is frequently found in combination with other congenital abnormalities; its particular association with mongolism is well known.

Aetiology

The pancreas is developed from a dorsal and ventral primordium (Fig 4) The dorsal anlage lies just abo the d ct choledochus and gi es rise to the formation of the entire pancreas, with the exception of the under most right quadrant of the head of this organ. The ventral anlage is made p of t ary

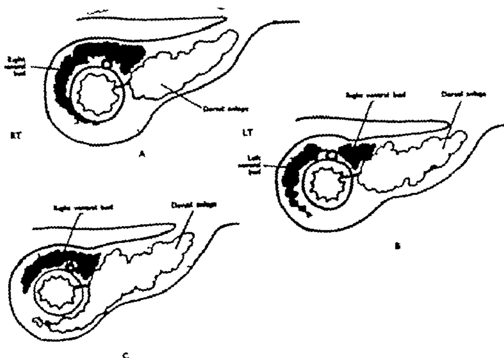


Fig. 5 Theories of development of annular pancreas. A. Lecco theory with the fixed top of the right ventral anlage B. Baldwin theory in which the left ventral anlage is retained and becomes larger C Tiekme theory with hypertrophy of the right ventral and the dorsal anlage (After Anderson & Wapshaw).

ments, each located on opposite sides of the ductus holedochus; the left segment usually becomes atrophic and disappears. Rotation f the intestines is accompanied by a movement of the residual entral anlage to the right and dorsalwards, ultimately fusing with the dorsal anlage forming the undermost right quadrant of the head of the pancreas.

At the present time, there are various theories concerning the formation of the annular pancreas (Fig. 5)

I According to Tiekm, the ring is supposed t be formed by the hypertrophy of the entral and dorsal segments of the head of the pancreas. These two segments approach each other and fuse t the right f the duodenum. This theory is supported by Brines, and Lerat. According t Lerat, the hypertrophy is a result of an inflammatory process.

II Lecco assumes that the top of the right segment of the ventral anlage becomes fixed. Due to the rotation of the intestines a band shaped organ is formed around the duodenum. This theory is in agreement with the fact that in the majority of the cases studied the excretory duct f the ring commences on the ventral side of the duodenum, running over this organ to the right and dorsalwards, and finally coursing over the dorsal surface to the left; ultimately emptying into the duodenum.

III. Baldwin suggests the possibility that in some cases the left segment of the ventral anlage is retained. This supposedly becomes larger in size, eventually giving rise to the formation of the ring.

IV The potential ability t form pancreatic tissue is still retained by the primitive intestine. Normally this potential is concentrated in the dorsal and ventral anlage. If this concentration is incomplete various

glands appear at the same level fusing at a later stage to produce the ring form pancreas. Erimoglu, who advanced this theory found, after studying a tissue preparation obtained from a 46-year-old man that in addition to the pancreatic duct, there were also four other smaller excretory ducts which ran separately from the ring to the duodenum.

Therapy

The necessity for surgical intervention will depend upon the degree of obstruction. In most cases, the diagnosis is not made preoperatively and the finding of an annular pancreas usually is a surprise.

In the course of time a number of different techniques have been employed.

1. Lerat, performed a partial resection of the ring with good results, while both Sillvis and Howard's cases were complicated by a pancreatic fistula.

The frequent occurrence of duodenal atresia at the site of the pancreatic ring imposes an additional disadvantage to this technique. At most, resort can be made in these cases, to a Heineke-Mickulicz plastic operation.

2. If the diagnosis is made at a later age, the condition is usually associated with an ulcus ventriculi, chronic gastritis and scar formation. In these cases, Cutter and Waugh resorted to partial gastric resection.

3. Posterior gastro-enterostomy was employed by Vidal, as well as by Bickford & Williamson.

4. In general, isoperistaltic duodeno-jejunostomy is considered to be the best technique (14). This method has the advantage that it prevents stasis in the portion of the duodenum above the obstruction.

Summary

A description of two cases of annular pancreas in infants which gave rise to complete obstruction of the duodenum is presented. Based on the literature and personal experience the symptomatology aetiology and therapy are discussed in detail.

References

1. ANDERSON J and WAPSHAW H. Annular pancreas. *Brit J Surg* 39 43 1951/1952.
2. BALDWIN W. A specimen of annular pancreas. *Anat Rec* 4 299 1910.
3. BALLINGER, J. Hypoglycemia from metastasizing insular carcinoma of aberrant pancreatic tissue in the liver. *Arch Pathol* 32 277 1941.
4. BICKFORD B and LLOYD WILLIAMSON J. Annular pancreas. *Brit J Surg* 39 49 1951.
5. BRINES, O. Annular pancreas involved in acute haemorrhagic pancreatitis. *Arch Surg* 92 41 1930.
6. CHAPIN J and MORRIS H. Annular pancreas accompanied by aberrant pancreatic nodule in the duodenum. *Amer J Surg* 60 788 1943.
7. CLATWORTHY J H and LLOYD J. Intestinal obstruction of congenital origin. *Arch Surg* 75 880 1957.
8. COHEN H. Accessory pancreas with ulcer of the pylorus. *Surg Gynec Obstet* 51 331 19..
9. CUNNINGHAM G. Annular pancreas. *Brit J Surg* 27 678, 1939/1940.
10. CUTTER, M and WAUGH, J. Annular pancreas with secondary dilatation of the duodenum. *Proc M[ayo] Clin* 19 33., 1944.
11. DANKER, M.: Aberrant pancreatic tissue in the gastro-intestinal tract. *Surg Gynec Obstet* 47 820 1928.
12. ECKER A. Bildungsfehler des Pancreas und des Herzens. *Z Rat Med* 14 354, 1862.
13. ERIMOGLU C. A case of pancreas annulare. *Proc K Ned Akad Wet Serie C* 45 1., 195..
14. GROSS, R. and CHISHOLM, T. Annular pancreas producing duodenal obstruction. *A[nn] Surg* 119 38 1944.
15. HOWARD N. Annular pancreas. *Surg Gynec Obstet* 50 533, 1930.
16. KITCHIN A. Intussusception due to ectopic pancreatic tissue. *Lancet* II 938 193..
17. LECCO T. Zur Morphologie des Pancreas annulare. *S B Akad Wiss Wien*, 119 391 1910.

18. Lehman, E.: Annular pancreas as a clinical problem. *Ann Surg* *115* 574, 1942.
19. Lecat, P.: Contribution chirurgicale à l'étude du pancréas annulaire. *Bull Acad Roy Med Belg* *24* 237 en 290 1910
20. McNaught, J.: Annular pancreas. *Amer J Med Sci* *185* 49 1933.
21. Otterdoom, H.: Annular pancreas. *Ned T Geneesk*, *1* 476 1953.
22. Rawitch, M. and Woods, J. A.: Annular pancreas. *Ann Surg* *131* 1118, 1950
23. Rickham, P. Annular pancreas in the new born. *Arch Dis Childh*, *29* 80, 1954
24. Shapiro, D. Dietrick, F. and Gellis, E.: Obstruction of the duodenum in the new born infant due to annular pancreas. *Pediatrics*, *9* 784, 1952.
25. Silvis, R.: Annular pancreas. *Ann Surg* *135* 278 193..
26. Swynnerton, B. and Tanner, N.: Annular pancreas. *Brit Med J* *1* 1078 1953.
27. Tiedemann, F.: Über die Verschiedenheiten des Ausführungsganges der Bauchspeicheldrüse bei dem Menschen und den Säugthieren. *Deutsch Arch Physiol*, *4* 403, 1818.
28. Vidal, E.: *Pancréas annulaire et atrophie* du duodenum chez un enfant de trois jours. Gastro-entérostomie postérieure Guérison. Dixhuitième Congrès de Chirurgie Paris, 745, 1905.

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CASE REPORT

Aortic Thrombosis and Aortic Medionecrosis in an Infant

by ANDERS MOBERG

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(Head: F Wahlgren, M.D.)

Aortic thrombosis—usually developing from arteriosclerosis—is, from the pathologist's point of view a fairly common occurrence in the adult. On the other hand, however aortic thrombosis in infancy is rare In 1956 Moberg & Reinand studied the literature in connection with two of their own cases and since then no further cases appear to have been published. Of the 4 cases then on record where the patient was under 15 no less than 11 occurred in children less than 3 weeks old. There is no clear explanation for this preponderance of cases in the neonatal period.

The etiology of the aortic thrombosis has varied in the different cases. In some cases the thromboses have originated in the umbilical artery (10) or in the ductus arteriosus (6) In a number of cases infection is considered to have been the basic cause (3 8, 13 15 19) and in one of Moberg & Reinand's cases there was medionecrosis of the aorta. In almost half of the cases however no definite cause has been established.

Boy born at term, weight 2420 g The mother was a Para II and had had a previous abortion. She was healthy during pregnancy The delivery was normal. During the first 24 hours of life the child was normal. He subsequently became increasingly listless, vomited repeatedly and lost about 400 g daily H was transferred to a children's surgical department where, during the sixth day of life, the entire intestines were X rayed for suspected ileus or pylorospasm. No changes of importance were observed. The patient continued to vomit and also developed diarrhoea of a slimy consistency During the time that the child was in hospital the pattern of symptoms changed continuously and was dominated by extreme meconium like vomiting Despite adequate therapy the patient remained in a shocked condition the whole time and gave the impression of being intoxicated. During the last two days of life there was fetid blood vomiting and dark brown to black excrement of a loose consistency

Laboratory tests showed that the hemoglobin, the white blood corpuscles and the differential blood count were all of normal value. Cerebral spinal fluid examination revealed nothing abnormal. Urine examinations were normal to begin with, but on the 15th day of life numerous red and white blood corpuscles appeared in the sediment. Further blood analysis showed a slight acidosis. Despite general shock treatment the patient died on the 19th day of life

The *post mortem* revealed no cardiac malformation. In the left auricle there was an almost pea-sized thrombus. At the inlet to the aorta the ductus arteriosus was thrombosed (Fig 1). From this point a pea-sized



Fig. 1. The thrombus in the ductus arteriosus extending into the aorta.

thrombus had penetrated into the aorta. Proximally this thrombus was lightly attached to the wall of the vessel.

In the abdominal aorta a large thrombus was visible (Fig 2). This extended from the outlet of the celiac artery down to the aortic bifurcation. It did not continue int the iliac arteries. It was firmly attached t the wall of the vessel and entirely occluded the outlets of the renal, and the superior and inferior mesenteric arteries. Both of the kidneys and a large part of the intestines showed hemorrhagic infarction.

The umbilicus was normal and showed no signs of infection. There were no thrombi in the umbilical veins In the umbilical artery



Fig The large aortic thrombus. Note the discoloration of the kidneys due to hemorrhagic infarction.



Fig. 3 Ruptures of the elastic lamellae. W igert lastic tissue stain. 130 magnification.

there was a thin, 3 cm long thrombus which was lightly attached to the wall of the vessel, and which did not entirely fill the vessel.

...re and there in the lungs there were a ...ber of almost rice-sized centres which th... macroscopicaly and microscopically proved t... be aspirated blood. No significant changes were ...bservable in the other organs of the bod...

Microscop... nvestigation revealed similar changes throughout the entire aorta. In the wall ...f the aorta and particularly in the media the elastic lamellae were separated by an amorphous substance with a positive mucoid colouring. Here and there the lamellae were ruptured, and in and around these areas there were strand... of cell deficient collagenous connectiv... tissue (Fig. 3 and 4). *In a few places in the aortic wall, small* transverse ruptures were ...sible and around these ruptures a few red blood corpuscles could be seen, but there seemed to be no actual dissecting aneurysm ...f the aort... (Fig. 3). The ruptures ...ust therefore ha... developed just before death. Direct ...nnec-tion between the ruptures and the aortic thrombosis has, however not been found on the slides. There was no sign of inflammation in the aortic wall and no changes in the vasa vasorum. In the heart there were a number of very small and entirely insignificant fresh thrombi in the trabeculæ of the left ventricle. The myocardium was, however normal.

The cause of the disease and death of the patient was thus thrombosis in the abdominal aorta with secondary circulatory failure in the intestines and kidneys.

Besides the thrombus in the abdominal aorta, thrombi were also visible in the ductus arteriosus, the umbilical artery, the left auricle and in the trabeculæ of the left ventricle. It is difficult to establish in which order these thrombi developed. From the circulatory point of ...iew it ...ould seem to have been the thrombi in the heart that were the primary ones. But the fresh ...p-pearance of these belied this, ...part from the fact that neither macroscopically nor microscopicall... c...uld any reason ...be f...und as t...



Fig. 4. Detail enlargement of Fig. 3 (see text). Weigert elastic tissue stain, 30 magnification.

wh the thromboses should have developed in these portions. It is more likely that the thrombus in the ductus arteriosus was the primary one and that fragment of this had broken loose fastened in the aortic bifurcation and here developed into the large thrombus. Similar cases of this type ha been described previousl (6). Both the macroscopic and microscopic pictures, however indicated that the thrombus in the abdominal aorta was older than the one in the ductus arteriosus. The microscopic investigation of the aorta revealed, in several places, hanges in the media and speciall hin the elastic tissue. These changes correspond w h those that are usually seen in aortic medionecrosis. Transverse ruptures were observed although not directl connected ith the thrombus. Despite his it seems probable that these ruptures, the aortic edionecrosis and the aortic thrombosis, ere directl connected. Similar cases of his type ha also been described in the literature (11).

It thus seems likely that the primary changes were aortic medionecrosis with secondary thrombosis in the abdominal aorta. It is generally known that thromboses can develop during the period of closure of the ductus arteriosus and the



Fig. 5. Transverse rupture. The arrow indicates few red blood corpuscles. Ladewig modification of he Mallory stain, 120 magnification.

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PROCEEDINGS OF PEDIATRIC SOCIETIES

Danish Paediatric Society

Meeting January 13 1960

J Vesterdal The Effect of Durabolin (19 nor-androsterolone) in a Case of Hypophyseal Dwarfing

The patient was a boy aged 10 years who had suffered from eosinophile granuloma from the age of two years. The disease gradually subsided but the following sequelae persisted deaf mutism, diabetes insipidus, adiposity and dwarfism presumably of hypophyseal origin. The boy was treated for 12 months with Durabolin (12.5 mg intramuscularly per week) during which time the rate of growth increased 9 cm compared with 4 cm the preceding year and 2 cm in the subsequent eight months. Marked increase in eight but only insignificant virilization were bserved a side-effects No definit acceleration of the development of the centres of ossification was observed.

DISCUSSION *E Thamdrup* May the increase in growth perhaps be an effect of testosterone? Dwarfism is treated as a rule with preparations of testosterone commencing at puberty — *Henning Andersen* A human growth hormone has been manufactured and appears to be effective but is not yet on the market. Durabolin is scarcely more growth-promoting than testosterone The employment of Durabolin in premature and cachectic children is not advised, because experiments on rabbits suggest that the preparation may have a sterilizing effect on females. — *P Plum* Physex (10 injections of 1500 units) produces increased growth and simultaneous commencement of puberty in some cases.

B. Friis-Hansen Anaemia, Malnutrition and Parasitic Infestations in African Children

(To be published lsewhere.)

B Friis-Hansen Vitamin A Deficiency and Blindness in African Children

(To be published lsewhere.)

Meeting February 10 1960

Sr Brandt Demonstration of Generalized Muscular Hypoplasi and Bulbar Paresis n an Eight-y r-old Boy

A boy ag l eight ears was demonstrated who since birth has suffered fro generalized muscula h poplasia. The condition resembled muscular dy trophy clinically bu t there had been no evidence f progression In addition, the boy showed signs f hypoplasia and weakness corresponding to the muscles innervated by cranial nerves VII, IX X, XI and XII Paralysis of the soft palate had been present since birth and had caused serious feeding difficulties so that the boy spent the first 1 years of his li in paediatric wards where he had to be tube fed for long periods. At present there are no feeding difficulties but speech is still affected by the paralysis of the soft palate and incompetence of the labial muscles This

combination of congenital hypoplasia of muscles with bulbar innervation (nuclear hypoplasia?) and generalized muscular hypoplasia is very rare On the other hand, both conditions occurring separately are well known although also rare Myasthenia gravis was excluded by a prostigmine test when the child was six months old. Muscular dystrophy appears to have been excluded by a biopsy from the vastus lateralis when the boy was 14 months old. Electromyography when 18 months old, showed short potentials (deltoid, tibialis anterior) and a tendency to fall-out in potential. (Short duration of potential is observed in polymyositis and in non-hereditary conditions which resemble muscular dystrophies. Both of these conditions appear improbable in the present case on account of the course and the biopsy findings.)

H Dyggi Congenital Ascites

Six cases of congenital ascites accompanied by generalized oedema and hydramnios but without blood type incompatibility are reported. The sixth case a male infant aged two months, is still in the Paediatric Department of the University Hospital. H probably has chylous ascites. On the second, eight and thirtieth days of life approximately 400 ml ascitic fluid were withdrawn by abdominal paracentesis. This fluid became increasingly milky and contained 0.5 per cent fat. A cystic swelling could be felt at the site of the gall bladder When the infant was five weeks old cyst of the liver containing chocolate-coloured fluid was removed at exploratory laparotomy Ascites has again accumulated. The treatment planned is repeated abdominal paracentesis and low-fat, protein-rich diet If this proves ineffective veno-peritoneal anastomosis (Routte operation) may be considered Thirteen cases of congenital chylous ascites which were cured by other operations have been described while only tw died.

Discussion *J Flemand Christner* I the case from Odense no thoracic duct was found and many of the lymph vessels in the intestine ended blindly Other operations have also been attempted and among these isolation of a small segment of the small intestine which is opened and sewn to the peritoneum has been effective

J H Probst Vitamin D-Resistant Rickets

A boy aged 14 years suffering from vitamin D-resistant rickets was followed for 12 years. The findings deviate from those accepted as typical in the somewhat early commencement (at the end of the first year of life) only slightly lowered or sometimes low to normal serum phosphorus and very low serum calcium (as low as 5 4 mg 100 ml). The alkaline phosphatase values were elevated and were normalized by adequate therapy The dosage of vitamin D necessary was between 50 000 and 250,000 units daily Hypercalcaemia (12.9 and 14.3 mg 100 ml) occurred for two brief periods. The symptomatology of this complication is discussed: polyuria, polydipsia, reduced renal concentration, slight azotaemia and slight sporadic pyuria and uncharacteristic cerebral seizures with slightly abnormal EEG all of which were completely reversible on normalization of the serum calcium by reduction of the dose of vitamin D. Normalization of the alkaline phosphatase values together with healing of the bone lesions and increased growth in height are considered to be the best indications of the efficacy of the treatment Hypercalcaemia is an indication of overdosage and raised alkaline phosphatase values for underdosage The occurrence of hypercalciuria prior to hypercalcaemia makes Sulkowitz test a valuable control measure The diminishing requirement of vitamin D with increasing age makes increased control necessary at puberty when overdosage may easily occur Corrective osteotomy should only be undertaken late and preferably when the patient is fully grown, it is useless before normalization of the bone lesion. There is a risk of hypercalcaemia following the osteotomy and, for this reason, vitamin D should be temporarily withdrawn during this period. Hypophosphataemia is regarded as a more sensitive indicator of the presence of the abnormal

gene than a manifest skeletal lesion. The mode of inheritance of the hereditary cases (approximately half of the cases recorded in the literature) is considered to be a sex linked dominant. Female carriers only rarely show bone manifestations and, if so, the changes are minimal. The sporadic form, to which the present case belongs, cannot be differentiated metabolically from the hereditary form but in this form, females may also have severe skeletal involvement. Current opinions concerning the pathogenesis are discussed.

Discussion *Sv Brandt* In orthopedic departments, surgical intervention is frequently undertaken on severely deformed bones. To what extent can growth correct the deformities so that surgical intervention may be avoided? — *J Flamand Christensen* had followed a similar case in a boy who had a serum calcium of 6.8 mg/100 ml at the age of two years and who had been hospitalized a year previously elsewhere on account of seizures. The serum phosphorus was not less than 4 mg/100 ml and the phosphatase 40 King-Armstrong units. Slight acidosis was present. Increased excretion of cystine and ...ine in the urine were found on repeated ...ino acid investigations. The child improved on treatment with 60,000 units of vitamin D daily with supplementary calcium and phosphorus. An uncle possibly also had the disease — *B Friis Hansen*. Bone deformities may be due to many other diseases and, therefore medical treatment should be attempted first before surgical intervention is resorted to. — *Henning Andersen*. It is surprising how much bone deformity may improve between the ages of 12 and 15 years and therefore osteotomy should not be undertaken prior to puberty except under special circumstances. — *J H Probst*. It is uncertain whether the sporadic and the familial cases are the same disease. Osteotomy should be undertaken only during periods of biochemical homeostasis and may then be of good effect. Otherwise patience must be exercised.

Bjørn Andersen & Folke Tudvad Attempts to Determine the Enzymatic Activity of the Intestinal Canal under Physiological Conditions

In the Children's Hospital, Fuglebakken, Copenhagen, attempts were made to measure the enzymatic activity in the intestinal canal particularly in children with dyspepsia. Short plastic tubes of suitable diameter containing either protein or fat were administered orally to a total of 22 patients. The tubes were recovered from the faeces and the amount of the substrate digested measured. Eleven children without or with only insignificant transient dyspeptic symptoms digested, on an average, 2.4 mg protein and 2.0 mg fat, while 11 children with dyspepsia of longer duration showed much greater digestion both of the protein and particularly of the fat. This returned to normal as the dyspepsia improved.

Meeting March 9 1960

M Osler The Body Composition of Newborn Infants of Diabetic Mothers

The body composition of new born infants of diabetic mothers was determined on the basis of investigation of the fluid content the fat content fluid distribution in intra and extracellular spaces and neonatal deviations in the fluid content the conditions of excretion in the urine and the maturity as determined by the development of the centres of ossification. The conclusions drawn from the investigations were that infants born to diabetic mothers must be described as infants whose maturity as expressed by the development of the centres of ossification, water and nitrogen excretion in the urine and the functions of the other organs, correspond to their chronological age or perhaps slightly less, i.e. they are premature. In addition, they are markedly overweight because of excessive fat and to a lesser degree carbohydrate depot in the tissues and organs.

The incomplete development of the function of the organs, fluid release on the breakdown of the carbohydrate depots following birth and adiposity which impedes respiration are considered to be of possible significance for the frequent occurrence of the neonatal pul monary syndrome in infants born to diabetic mothers. Insulin, particularly in combination with copious administration of carbohydrate increases the fat and carbohydrate depots without influencing the maturity whereas growth hormone reduces the fat content. It must therefore be presumed that the macrosomia of diabetic hildren is due to increased supply of glucose form the mother with subsequent increased insulin production in the foetal pancreas.

DISCUSSION *B Friis-Hansen* W probably harm these infants by putting them in incubators with relatively high humidity which impedes their fluid expenditure. — *H Dyggve*. It has been stated in the literature that the incidence of the hyaline membrane syndrome is reduced when glucose is administered. If infants develop respiratory difficulties, they must have oxygen but attention must be paid to the degree of humidity and the body temperature should not be reduced too much. The target should be 35–36°C — *M Osler* The time of delivery should be adjusted, bearing in mind the facts that full-term births result in more deaths in utero while too premature births result in more cases of the respiratory distress syndrome. The great nitrogen excretion is due to breakdown of protein. Seizures were described in approximately half of the infants and these were probably due to low serum calcium. By and large the excretion of electrolytes was great. Excretion of calcium may perhaps increase the excretion of potassium. — *Jørgen Pedersen* W have demonstrated that these infants do not have hypoglycemia and no definite effect could be demonstrated from administration of glucose. Care should probably be exercised in the administration of fluids One third of the diabetic mothers were delivered by Caesarean section with low infantile mortality

J H Probst Two Cases of Water Intoxication

CASE 1 The patient was a boy aged four months with congenital megacolon. The infant was admitted in a state of marked dehydration (serum Na 164 mEq/l) unconscious and with a distended abdomen following gastroenteritis. Despite careful rehydration and repeated lavage of the intestine in the course of the night with transient improvement, the infant developed an attack of generalized prolonged tonic seizures 18 hours after admission. The child was comatose with bilateral Babinski reactions; stimulation by touch triggered the seizures. The cerebrospinal fluid, serum calcium and bicarbonate were normal. It was then discovered that the intestinal lavage had been undertaken with tap water and that the return was negligible The serum Na was 121 mEq/l and the total osmotic concentration 270 mOsm/l. One hour after administration of hypertonic mannitol, complete clinical remission occurred despite falling sodium values but increasing osmotic pressure The infant smiled, could fixate and suck and the plantar reflex was normal. The improvement continued and, following a massiv diuresis, the serum values returned to normal. EEGs recorded one and seven months later were normal.

CASE 2 The patient was a boy ged four years with a fractured pelvis and slight cerebral injuries following a traffic accident. During the first day there was pronounced oliguria with a specific gravity of 1 022 and catheterization was necessary Gastric suction was instituted on account of vomiting and abdominal distension. Intravenous ther py was instituted on the fourth day Five per cent glucose solution was administered in quantities of 1200 (750 ccall) and 1950 ml on the fourth and fifth days. Generalized tonic-clonic seizures occurred suddenly in the middle of the fifth day The Babinski reaction was present and the hild lost consciousness. Thereafter numerous seizures followed. Examination of the ey grounds was normal The CSF was also normal.

Serum chloride was 75 mEq/l. An unsuccessful attempt was made t control the seizures with massive intravenous administration of chlorpromazine, nembutal and pen tothal. Respiratory difficulties necessitated tra heotomy on the fifth day Shortly before transfer to the University Hospital the intra venous fluid therapy was changed to glucose-saline This was continued as saline and the barbiturates were withdrawn. There were no further seizures and the diuresis in the subsequent 24 hours was 1750 ml and the boy's condition inproved. During the subsequent three days, he showed a total negative fluid balance of over 2000 ml corresponding to 18 per cent of the body weight The serum values returned to normal

In addition t the diagnostic difficulties in a case of seizures in a child with the possibility of cerebral injury the explanation here is probably that such an overdosage of water (3 % glucose) occurred because the fl id balance was recorded on a hart for adults. This chart had a printed value for fluid loss by perspiration of 1500 ml while the calcu lated value for the boy was 560 ml and thus an excessi e dosage of one litre was adminis tered. The antidiuresis was primarily post traumatic and later probably maintained by the massive doses f barbiturates which prevented possible homoeostatic self-regulation. Replacement of the loss of electrolyte-con taining fluid by 5 % glucose sol tion f urther accentuated the hypotonicity

Crawford Dodge & Probst demonstrated by experimental studies on rabbits with bal ance experiments, EEG and brain analyses that the essential lement n water intoxi cation is hypotonicity of the body fluids with subsequent intracell lar oedema. The symptoms of water intoxication are neuro logical, a the brain cells are particularly sensitive to oedema and perhaps mostly because they re enclosed in the rigid cra nium. Damaged cells are particularly vulner able Fre quentl seiz res occur with a too rapid ret rn to normal val ues. The clinical ymptoms of water int oxication include increasing louding of conscio sness, ad nausea and path nd the pat ent re frequently pale, cold and sweating ("pseudo-shock"), but the blood pressure and pulse are normal until terminally Great quantities of dilute urine are voided. Spasms and tremor occur and the Babinski reaction is present. Later generalized tonic-clonic seizures oc cur Barbiturates have a transient effect. The coma becomes deeper and ends fatally in the untreated cases. The criteria for hypotonicity are the low serum electrolyte values or even better reduced serum osmotic concentration The EEG shows greatly reduced amplitude, practically absent normal rhythm and dominance of abnormal slow frequencies. Treatment consists of administration of a hypertonic solution: 3 % sodium chloride, 15 % mannitol or 6 % urea with 5 % glucose with the object f increasing the tonicity by approximately 20 mOsm/l in the total body fluids. By choosing the above-mentioned fluids, all of which remain extracellularly the correct fluid distribution is ensured with resulting dehydration of the ce lls. In the rabbit experiments and in Case No. 1 it was demonstrated that improvement occurred during mannitol therapy although the sodium and chloride values in the serum continued to fall, thus proving that the tonicity is the decisive factor In milder cases, particularly when no serious cerebral symptoms demand immediate therapy the patient may be made t dehydrate himself by reduction of the amount of fluid administered.

DISCUSSION *B Friis Hansen.* During the days immediately after cardiac operations, patients ha e a tendency t tremor In t e cases my colleagues and I found hyponatriaemia which w interpreted as water intoxication. Fifty per cent glucose solution ad ministered i travenously produces an almost immediate effect on oedema of the brain. — *P W Braestrup* Water intoxication is probably much more common than is assumed I have also seen a case in a child who had just undergone a cardiac operation and he had EEG changes and seizures. — *Henning Andersen* Lavage of a megacolon with isotonic saline is not entirely satisfactory either W now em ploy 7 % gelati solution

H J Probst. The mechanism of the development of the symptoms in cardiac patients is unknown; it may be due to sodium loss. The clinical picture corresponds to that encountered in water intoxication and many of the cases described as shock may have been cases of water intoxication. The symptoms may develop in the course of 20 minutes. Gelatine solution is excellent but cannot be employed in the presence of barium. Saline lavage may be undertaken once or twice without untoward results.

Paul Anthonisen, O Sennicke & Aage Thomsen A Case of Thrombocytopenic Purpura Complicating Infectious Mononucleosis

After a few days with symptoms suggestive of acute leucosis with swelling of lymph glands, enlargement of the spleen, epistaxis and haemorrhage from the mouth, a boy aged seven years developed pronounced purpura of the skin and macroscopic haematuria. The increased haemorrhagic tendency was caused by a low thrombocyt count in the blood (minimum 2000/mm³) The haemorrhages were so massive that signs of impending circulatory shock developed. This was corrected with blood transfusions. One week after the onset of the illness, the boy developed violent tonsillitis with membrane formation. Blood smears t this time and retrospectiv examination of previous preparations revealed the characteristic picture of mononu leosis Evidence of liver involvement (raised thymol reaction, serum transaminase and high gamma globulin values) was also present. The Paul-Bunnell reaction was negative or doubtfully positive but this is not evidence against the diagnosis. After the elapse of three weeks, the boy felt quite well again.

Review of the literature revealed only *6 corresponding cases. The cause of thrombocytopenia in mononucleosis is unknown The case described was treated with prednisone although, according to the literature there is no evidence that steroid treatment has any effect The prognosis is good.

Meeting April *0 1960

F Tad ad paid tribute t the memory of the late Dr Axel Friedlænder

H. Krentzfeldt Continued Experience with Triple Vaccine

The results of experiments with prophylactic vaccination with triple accine (diphtheria–tetanus–pertussis vaccine) under taken on 633 children in series of institutions are presented. The protection against whooping cough was satisfactory and complications connected with the vaccination do not appear to have been greater in extent than those which may occur following accination with diphtheria–tetanus vaccine It is therefore recommended that generalized prophylactic vaccination with triple vaccine be introduced from the age of 4–5 months. In addition, the indications and possibilities for the introduction of vaccination with pertussis vaccine alone are discussed.

St. Tulinius Experience with Pure Pertussis Vaccine

Three doctors, in co-operation with the National Serum Institute administered pertussis vaccine prophylactically t the infants in four infant nurseries and three hild-welfare centres. In the child welfare centres triple vaccine was occasionally employed after the age of fi e months. In the nurseries triple vaccine was only employed in a few cases.

Of 431 children in the four nurseries vaccinated against pertussis, 65 were exposed to infection prior to the age of five months and only one of them contracted the disease The material from the child-welfare centres does not permit evaluation of the effect of vaccination as the exposure is unknown and it is not known with certainty how many developed whooping cough.

In connection with the vaccination redness

and induration occurred at the site of vaccination and attained a maximum in the course of 6–7 hours and many of the infants were restless and fretful for a couple of hours about this time No high temperatures were recorded. In isolated cases, the mothers stated that the children were irritable for a couple of days. No abscesses at the injection site nor generalized hypersensitive reactions occurred.

It is concluded that pure pertussis vaccine may be employed with advantage from the age of five weeks, that the side-effects are minimal and the effect is apparently good

DISCUSSION *H Kreutzfeldt* Prophylactic vaccination with triple vaccine may probably be carried out within the existing legal programme with the amendment that an extra vaccination must be given. On the other hand, free vaccination with pure pertussis vaccine would require a special paragraph in the law The National Serum Institute and the Ministry of Health might consider that an attempt be made to carry out free vaccination with triple vaccine but to run parallel udies with pure pertussis vaccine in three f children: (1) those exposed to in on to determine therapeutic effects; (2) institutionalized children under the age of four years; (3) other children under the age of four months at the parents request. It would be desirable if the National Health Insurance could pay for vaccination for group (1) the Ministry for Social Affairs for vaccination for group (2), while those who seek vaccination in group (3) must defray the cost themselves. — *Sv Tulinius* Triple vaccination cannot be commenced prior to the age of five months as the effect of diph theria–tetanus vaccination is not therwise achieved. I am therefore in favour of the programme outlined by Dr Kreutzf ldt but would not recommend booster injections, as it is desirable that as many children as possi ble have a mild attack f whooping cough in childhood Booster injections may delay whooping cough until adult lif — *P W B estrup* It would be desirable t carry out early pertussis vaccination in all h ldren but the practical difficulties, including the establishment of new laws, are probably too great. In addition, there is a risk that people as a whole, become tired of vaccination and that it becomes difficult to record all the various vaccinations. — *J Flamand Christensen* What would happen if a single pertussis vaccination was administered t the prophylatic health examination at the age of five weeks followed by triple vaccine at the age of five and six months? Can polio vaccination administered simultaneously with triple vaccination be expected to produce the same good antibody formation as previously? — *P Plum*. Will whooping cough not disappear as vaccination is gradually carried out so that there is no longer such a danger for new born infants? — *O Fridericksen* Is it possible that new-born infants who, for example, are exposed to whooping cough in their homes can be protected with four or five injections at short intervals? Can pertussis vaccination be continued in a child who has had convulsions in connection with vaccination? — *Sv Tulinius*. It is scarcely correct to give pertussis vaccination alone at the age f five weeks and continue with triple vaccine at the age of five and six months. A good increase in the antibody titre for pertussis would probably occur but there is a risk that the increase in the diphtheria and tetanus antibodies is poorer Polio vaccination may well be administered simultaneously with tripl vaccination. It is scarcely probable that whooping cough will disappear even when vaccination has been carried out. A number of mild cases of the disease will probably still occur which are, however just as infectious. Protection may be anticipated by pertussis vaccination of new born infants, which, however rapidly disappears. — *K Willen-Jense* Experience in vaccination of asthmatic patients does not suggest that more antigens reduce the antibody formation for one or more of the antigens. — *I Scheibel*. Triple accine should not be administered prior to the age f four to five months as too many infants have too many antibodies in the blood as a result of diphtheria vaccination in the mo-

ther These are not excreted until after pproximately 20 weeks of age and thus the full effect of vaccination in the infant cannot be expected until after this time. — *F Bruhn-Petersen* Has any attempt been made to protect infants from whooping cough by vaccinating the mothers during pregnancy? — *Sv Tulinius* As far as I know this has never been attempted and, personally I would not recommend it on account of the risk of marked reactions in the pregnant woman and possible injury to the foetus — *Asa Madsen* At the child welfare centres only a few mothers did not desire pertussis vaccination. — *Torben Iversen* Does the National Serum Institute still recommend therapeutic vaccination (several injections at short intervals) to children exposed to whooping cough? — *S Tulinius* The National Serum Institute no longer recommends this form of vaccination. It must also be pointed out that the Institute cannot at present supply triple vaccine for purposes other than the experimental work at present in progress. At least eight months preparation will be required before the vaccine is generally available — *F Tudvad* As there seems to be unanimity as regards the desirability of altering the hitherto employed diphtheria-tetanus vaccine to diphtheria-tetanus-pertussis vaccine, the Danish Paediatric Society will ppeal to the Ministry of Health in this respect and recommend that permission be given for administration of four vaccinations free of charge

Bjørn Andersen & Kjeld Nielsen Serum Proteins in Premature Infants

In the Children Hospital Fuglebakken, the variations in both the total serum proteins and the various fractions, were investigated in 39 premature infants who did not present any clinical symptoms apart from prematurity The birth weights ranged from 1100 to 2500 g. Both longitudinal and group investigations were undertaken and the results were in agreement Normal curves for the bsolute alues of total protein, albumin and gamma globulin are established in group investigations of premature infants fed exclusively on breast milk. The total protein and albumin values fall from the first day of lif until the age of five to six weeks and thereafter rise again until the age of ten weeks. The gamma globulin values fall steadily during the first ten weeks.

In group investigations in premature infants who had received the same quantities of breast milk plus supplementary protein in the form of 1½ % casein-hydrolysate (Idon) increased values for albumin and total protein were observed during the first ten weeks, although this group experienced the same fall as premature infants fed on breast milk alone On the other hand, reduced gamma globulin values were found throughout the same period. It thus appears that supplementary protein added to breast milk with the object of accelerating growth produces in creased total protein and albumin in the serum but decreased gamma globulin values.

Kjeld Nielsen Two Cases of Tumours f the Lung in Children

Two cases of tumours of the lung admitted to the Children s Hospital, Fuglebakken, within a year are presented.

The first case was a girl aged 10½ years who had suffered from pyrexia and slight cough for more than three weeks prior to admission in February 1950 X ray of the lungs showed infiltration superiorly in the right lung with enlargement of the hilar glands. This remained stationary for three months. Tomography which raised the suspicion of pulmonary cysts, revealed that the infiltrations were situated posteriorly in the right lung. There was a positive tuberculin reaction following previous Calmette vaccination. Culture from the stomach washings proved negative for tuberculosis. The blood sedimentation rate was initially elevated but later returned to normal. The cold agglu tinin and ornithosis serum reactions were negative as were the skin tests for fungus di seases Because the diagnosis was uncertain, the girl was treated with antituberculous chemotherapy for a month after transfer t the Department of Thoracic Surgery in Ore sundshospitalet. At subsequent operation, an

elongated hard tumour the size of a Brazil nut was found extending towards the right hilus. Microscopic investigation revealed mesodermal cellular undifferentiated tumor tissue probably a neurofibrosarcoma.

The second case was a girl aged four years who had suffered from uncharacteristic lassitude with slight morning cough for two months prior to admission. A half-sister aged eight years was found to be Moro positive at routine examination. On examination of the family at the Central Dispensary for Tuberculosis, a solid infiltration was found in our patient in the left lung. She was Moro negative and remained so during the eight-week period of hospitalization. No tubercle bacilli were found on culture. The cold agglutinin and ornithosis reactions were negative. At operation in the Department of Thoracic Surgery Oresundshospitalet, a cyst the size of a walnut was found in the paravertebral sulcus with a thin neurovascular pedicle attached to the medial part of the fourth intercostal space but unconnected with the sympathetic trunk or the spinal cord. Microscopic examination showed fibrillary connective tissue in the wall of the cyst with normal glands and several layers of cylindrical epithelium on the inner surface. The cyst was probably bronchogenic.

Meeting May 22 1960

A Sell Embryological Investigations of the Human Alimentary Canal

A revised conception of the development of the alimentary canal based upon investigations of approximately 500 human foetuses is given. Development takes place in an oral-anal direction. The duodenum develops partly from the most proximal part of the foregut and partly from the most proximal part of the midgut. The part of the duodenum which originates from the foregut, develops very early and leaves the midgut between the postarterial midgut segment and the hindgut, so that the small intestine migrates from the right to the left side of the abdomen. Simultaneously the intra-umbilical coils of intestine are "lifted" into the abdomen. In contrast to previous conceptions, it was demonstrated that the last part of the small intestine to come into the abdomen from the umbilical hernia is the portion immediately proximal to the terminal coils of ileum (corresponding to the transition between the pre and postarterial sections of the midgut) rather than the caecum along with the terminal coils of ileum After migrating into the abdomen, the caecum lies at the level of the umbilicus and is thereafter displaced over to the right side of the abdomen but still remains on a level with the umbilicus on the anterior surface of the inferior pole of the right kidney At this site, the rotation (total of 270°) of the caeco-ascending colon is completed at the same time as it descends into the right iliac fossa. The colon continues to grow in length the flexures form and, finally the mesenteric attachments are established.

Isolated pathological findings encountered in the foetuses examined are mentioned. Several cases of dystopia coeci proximo-lateralis at various stages of development were found This developmental anomaly is probably due to the fact that the caecum and the terminal coils of intestine are the last to come in from the physiological small intestinal hernia and are prevented from rotating and (therefore?) are abnormally situated. The earlier theory of cessation of development at a stage where the caecum lies up under the liver can no longer be accepted, as the caecum is never found so high at any stage in the normal development. Finally the conditions in malrotation are mentioned From embryological investigations compared with radiological findings, it is considered possible to postulate the hypothesis that malrotation (non-rotation) of the intestine may be due to incomplete development of the oral-duodenal segment, so that the normal development, outlined above does not occur In cases of malrotation, it has been found that

the bile duct opens bnormally close to the pylorus immediat ly distal to the bulbus duodeni. Finally cases of cascade stomach, coeco-ascendens breve, omphalocele and colon elongatum are mentioned.

E. Vad Preliminary Experience with Air Ionisation

Some examples of conditions caused by atmospheric electricity such as Föhn and mountain sickness are mentioned. The number of ions in the air amounts to approximately 700–800 per cc. The number depends upon the relationship between the rate of destruction and the rate of formation. The ions are formed by radioactive, cosmic and ultraviolet radiation. Certain observations suggest that there is a biologically optimal concentration and an optimal proportion between positive and negative ions. Passive increase of the ion level may be achieved by being certain that the ions formed naturally are given as good conditions of existence as possible. Active increase is achieved by various forms of ion generators. A follow-up investigation f 115 patients with asthmatic respiratory difficulties treated with ion therapy (Florsion) revealed that 62 per cent had improved but only 41 per cent to such an extent that it was of practical therapeutic significance Forty five of the patients were under the age of 15 years. In this age group, improvement occurred in 69 per cent and considerable improvement in a total of 52 per cent. Finally some experiments with patients placed on highly insulated beds are mentioned. It is possible to demonstrate intake of electricity from the air corresponding to a current strength of 10^{-a} t 10^{-} A, when a potential of 4000–5000 V is pplied to the patient The strength of the current increases if an ion producer is placed in the icinity of the patient The increase depends upon the strength of the ion producer and whether the patient faces the ion producer or not

DISCUSSION *S H ild* Many patients report sense of ell-being following radiation with ultraviolet light Is this due to increase of ionization? *P Bechgaard* Dr Vad has not drawn any conclusions from the investigations but, after having followed Bach s work with the Florsion pparatus we must state that we know too little about the significance of the electric conditions for man. We will now attempt to undertake measurements in this respect. It has been demonstrated that some patients are able to become charged with lectricity to an unknown extent and they feel uncomfortable. Such individuals should avoid nylon clothes etc. W have been able to cause dyspnoea by alteration of the ionization of the air but our knowledge of the whole subject is in its infancy — *E V ad* The effect of mercury quartz light may by and large be attributed to ionic action. Ion therapy is most effective when the patient is grounded. — *Engineer Bach.* Many individuals may become harged with static electricity so that long sparks form. In a room without green plants, with dry air plastic paint on the walls, floor and furniture an individual may become harged with 50,000 to 100,000 volts by walking across the carpet in leather shoes. I have investigated the humidity of such a room and demonstrated that 150 kg of water were necessary to establish the normal humidity Many people do not feel comfortable in such rooms. Antistatic treatment of the furniture floor and walls may improve conditions, as this treatment permits normal discharging to take place.

G Gregersen A Fifteen-year Material of Meningitis and Follow-up Examination from a Central Hospital

The material consists of 735 patients admitted to the Central Hospital in Randers during the period 1945–1959 Forty two per cent of all of the cases were purulent. The majority of the patients were hildren under the age of 15 years. Follow-up investigation was undertaken by sending questionnaires to the 447 patients who survived during the last ten years. Replies were received from 89 7 per cent Permanent sequelae were found in 24 per cent of the cases of meningococcal meningitis, 77 per cent of H influenzae and 54 per cent of pneumococcal meningitis. If

paralyses due to poliomyelitis were excluded, sequelae were found in 29 per cent of the cases of serous meningitis. Serious sequelae were concerned in only a small percentage. The most frequent findings were

	Purulent %	Serous %
Headache	12	8.4
Vertigo	4.5	7.2
Impaired hearing	9	1.[illegible]
Reduced mental capacity or delayed development	10.5	2
Irritability and neurasthenic complaints	12	10

No definite difference in the incidence of the sequelae was found in the various age groups. On the other hand, the incidence of sequelae was increased when the sugar in the CSF was less than 20 mg % or when pyrexia had been present for more than six days prior to admission.

DISCUSSION. *H. Dyggve*: Meningitis frequently begins insidiously in new-born infants and there are relatively many sequelae. Of *0 cases of meningitis in new-born infants diagnosed at the University Hospital in the past ten years, eight died and four developed hydrocephalus. Seven of the patients were premature. The meningitis was due to *B. coli* in eight cases and of these four patients survived but three had hydrocephalus. I would recommend that treatment with streptomycin, sulpha preparations and tetracyclines be commenced as soon as the diagnosis of meningitis is established in a new-born infant rather than wait for a bacteriological report.

Folke Tudvad, Copenhagen

BOOK REVIEWS

E. Denhoff and Isabel Robinault Cerebral Palsy and Related Disorders. A developmental Approach to Dysfunction.

McGraw Hill Company London, 1960 Four hundred and twenty-one pages. Price 93 s.

The book contains a comprehensive and thorough review of the rapidly accumulating literature on cerebral palsy as well as of the authors own vast experience on the subject. It is stressed that the condition is but one neuromotor aspect of brain-injured child and cannot be isolated from other cerebral dysfunctions such as mental deficiency epilepsy the hyperkinetic-behavior disorder and the sensory disabilities. The developmental pproach to these disorders implies that the manifestations may change as the child develops and these may have prognostic consequences. For example the hyperkinetic behavior syndrome tends to disappear with maturation Although the patient neuromotor handicap frequently persists the dynamic approach which is described by the authors forms the basis for the evaluation and treatment of those children, as practiced in the Meeting Street School in Providence. The associated sensory and perceptual-motor disturbances are dealt with in great detail and this chapter is especially instructive *for* those *regarding* cerebral palsy more or less as a simple orthopedic problem. However no matter how well these *cerebral-palsied* children are taken care of, the challenge of today is the poor social prognosis even for those with normal intelligence and *only* a *moderate* motor handicap Emotional and psychological factors apparently play an important role in this respect with *ensuing difficulties* for the oung adult in finding place and an occupation in the community or at least a meaningful existence Educational and vocational guidance are apparently not sufficient as reflected in the low rate of employment The monograph can be wholeheartedly recommended as a most useful source of information for all categories working in the cerebral palsy team.

Bo Hellström Stockholm

Ed by A. Hottinger and H Berger: Modern Problems in Pediatrics, Vol. VI. Renal Function and Kidney Disorders in Childhood.

S. Karger A.G Basel, 1960 560 pages. Price S.F J.—

The new volume in this series consists of 27 papers dealing with current problems in renal physiology and pathology with special emphasis on the pediatric age group Most of the authors are well known authorities in the field The purpose of the articles is either to review the most recent investigations or to focus attention on a limit d aspect of a particular topic. No original communications are presented. It is not *feasible to* review all papers in this book. If only some are mentioned it is because they hav been of special interest to the *reviewer* McCrory discussing glomerulonephritis in childhood, closes his paper with a very concise—and *from* the clinical *point of view* valuable—delineation of the obscure disease chronic nephritis. The overwhelming literature on the *nephrotic syndrome* is now almost impossible to follow However hretchmer Barnett & Ehlbuya present the essentials of *recent* investigation in this *field* and stress the modern concept that the nephrotic syndrome is not disease but a group of symptoms, which may appear in a variety of situations. Linneweh writes briefly and elucidatingly about acut pyelonephritis, especially the oligosymptomatic ariety of

useful guide to the clinician, particularly since each is written by an author with long-standing experience in his subject.

Like other volumes of *Advance in Pediatrics*, the eleventh is written so that it can also be understood by the physician who is not too familiar with the particular field at hand.

G Wallgren Stockholm

Fran Schmid and Helmut Moll Atlas der normalen und pathologischen Handskelett entwicklung.

Springer Verlag Berlin, Göttingen Heidelberg, 1960. Price: 8 DM.
10 tables, 115 fig 114 pp

This book is composed of two parts, an atlas and a clinical-diagnostic study. The atlas, which contains no less than 70 reproductions, forms the basis for the estimation of normal and abnormal values for the rate of appearance size and configuration of the skeletal elements of the hand. It is richly supplemented by tables, which present the normal variations in size of the carpal bones, as well as height and weight of normal subjects from the neonatal period up to 14 years of age. Additional tables give a condensed survey of the appearance time for the carpal bones and the epiphyseal centers of the hand, and the relation between the height of the individual and the length of the hand. In two tables the length of the humerus, radius and ulna is related to age and height of the normal individual. The relationship between the size of the carpal bones and height of the body is also given.

In the second part, an extensive account is presented of the diagnostic significance of abnormalities in the different bones of the hand as to number order and rate of appearance, configuration and disturbances of mineralization. This section is also richly illustrated. The reproductions are without exception of high quality. In a particularly interesting chapter the reflection of disturbances in the central nervous system on the skeletal development of the hand is briefly described. Deviations from normal are common and some of them have diagnostic significance. It follows that such patients are not feasible subjects for studies of normal variations in skeletal development, a point on which enough emphasis has not invariably been placed in earlier treatises.

According to the present study radiographic examination of the hand with due consideration to its length, the size of its bony elements and their rate and order of appearance should obviate the need for more extensive radiologic examinations of the extremities for determining skeletal age in daily practice. It is obvious that this means, among other things, a clear reduction in radiation dose.

In the reviewer's opinion, this book is an outstanding contribution to a subject that interests paediatricians and radiologists alike. It can be warmly recommended.

Ulf Rudhe, Stockholm

ANNOUNCEMENT

The X International Congress of Pediatrics

The X International Congress of Pediatrics will be held in Lisbon, Portugal Sept. 9 to 15, 196? with Professor C. Salazar de Souza as President. An excellent scientific program is being arranged with translation services for French and English. An extensive social program is planned as are scientific exhibits, industrial exhibits, and exhibitions of art and children's literature. The address of the Secretariat: Av. ?? de Maio, Lisbon, Portugal

Acta Paediatrica 50: 101–112 March 1961

From the Public Health Laboratory Manchester and the Streptococcus and Staphylococcus Reference Laboratory Colindale London

Further Observations on the Bacteriology of Impetigo and Pemphigus Neonatorum

by M. T PARKER and R. E. O WILLIAMS

The two organisms most often considered as possible causes of *impetigo contagiosa* are *Staphylococcus aureus* and *Streptococcus pyogenes* They have been isolated in widely varying proportions from series of cases examined at different times and in different parts of the world The idea that there are two clinical forms of the disease one staphylococcal and the other streptococcal, was first put forward clearly by Engman (1901). He considered that the classical *impetigo contagiosa* of Dunn (1863) and Fox (1864) which is characterised by a relatively thick stuck-on crust was a streptococcal disease and described in addition a bullous type from which *Staph aureus* could be isolated, usually in pure culture The close resemblance between the bullous type of impetigo and *pemphigus neonatorum* was also noted many years ago and several workers considered that they were essentially the same disease due to strains of *Staph aureus* with a special ability to give rise to an intraepidermal vesicular lesion (1 5 15) but numerous attempts to show that they differed culturally from *Staph aureus* strains from boils and abscesses were unsuccessful (9)

In 1955 Parker Tomlinson & Williams described an epidemic of impetigo in Salford, Lancashire in a which *Staph aureus* was isolated from 83 % and *Str pyogenes* from 47 % of the lesions. They found that three-quarters of the staphylococci belonged to a single phage type (Type 71) which was not commonly found in other sorts of lesion and was rare in deep suppurations. Also, 78 % of the strains of *Str pyogenes* gave one of two patterns. 3/13/B3264 and 5/11/12/27/44 when typed by agglutination. In the same year Barrow (3) examined a series of cases of impetigo in Bradford and found that 63 % of the *Staph aureus* strains belonged to Type 71 He obtained *Str pyogenes* from only 17 % of his cases and only one third of them had the typing patterns commonly observed in Salford. His cases had been seen in a hospital out patient department whereas the Salford cases had been swabbed in school minor ailment clinics. Spittlehouse (22) examined swabs from a series of hospital out-

patients with impetigo in Sheffield during the following year and also noticed a predominance of *Staph. aureus* Type 71 but he gave no information about the isolation of streptococci.

Although *Staph. aureus* Type 71 did not predominate in any lesion other than impetigo the Manchester workers noticed that it was found fairly often in bullous and pustular skin infections of newborn infants, and in 1957 Gillespie, Pope & Simpson described a hospital outbreak of *pemphigus neonatorum* due to it.

Type 71 staphylococci have a number of unusual cultural characters. They inhibit the growth of *Corynebacterium diphtheriae* in surface cultures on blood agar with the production of a characteristic sharply demarcated zone, and they produce opacity on serum agar but not in an egg yolk medium (17). The use of these tests has made it possible to recognise a small number of other strains with '71-like' characters. They too are found exclusively in superficial situations, and are associated with impetigo and *pemphigus neonatorum*.

It is tempting to believe that *Staph. aureus* Type 71 and *Str. pyogenes* types 3/13/B3264 and 5/11/14/27/44 are the causes respectively of staphylococcal and streptococcal impetigo. The significance of the very common mixed infections in which both *Staph. aureus* and *Str. pyogenes* are found in the same lesion has still to be explained. In the present paper we summarise information obtained on the bacteriology of impetigo in south-east Lancashire in the years 1953–7 and compare the frequency of occurrence of certain staphylococcal and streptococcal types, alone and in combination, in cases seen in school clinics and in a hospital out-patient department. We also give further information about the association of *Staph. aureus* Type 71 with infections of newborn infants, including *pemphigus neonatorum*.

Materials and Methods

Between September 1953 and August 1957 swabs from 514 cases of impetigo in children were received, nearly all from school clinics, 403 of them in Salford and the rest in neighbouring towns. Also in the years 1956–7 95 patients diagnosed as impetigo in the Out-Patient Department of the Manchester & Salford Skin Hospital by Dr J. K. Morgan and Dr K. R. Haye were swabbed when they first attended at the hospital. All swabs were cultured on blood agar and on gentian violet blood agar and strains of staphylococci and streptococci were isolated and identified by methods described previously (19). During the 4 years from September 1953 532 strains of *Staph. aureus* from lesions of newborn infants, mainly but not exclusively infected in hospital, were collected.

Strains of *Str. pyogenes* were typed both by the slide agglutination (14) and by the precipitation (23) methods. Very few of the streptococci were typable by the M precipitin test and many gave complex agglutination patterns with T sera for several types. A strain described as having, for example, the agglutination pattern 3/13/B3264 was one which was agglutinated by two or more of these T sera but gave no M precipitation reaction.

Strains of *Staph. aureus* were phage-typed as described by Anderson & Williams (2), using the basic set of phages recommended by the International Committee on Phage Typing (Report 1959), except for phage 80, which was only included after August 1955. They were also subjected to the following tests: penicillin resistance, inhibitory action on *C. diphtheriae*, the egg yolk reaction, and the opacity reaction on horse serum agar as described previously (17).

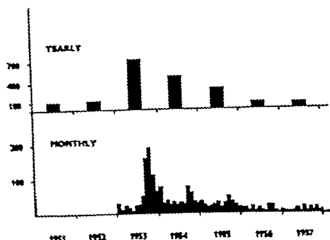


Fig. 1. Number of cases of impetigo treated in school clinics in Salford.

Results

Impetigo was found to be unusually prevalent among school-children in Salford when the schools re-assembled after the summer holidays in 1953. Many of the cases were exceptionally severe and were characterised by the presence of large bullae. Lesions occurred not only on the face and neck, but also on the chest, abdomen, buttocks, hands and arms (4). At about the same time it was reported from the neighbouring borough of Eccles that an unusual bullous skin infection had become common among school-children. The information in Fig. 1, which shows the annual and monthly numbers of cases of impetigo treated in Salford Health Department clinics, was provided by Dr J. L. Burn. In his opinion, the figures for the peak of the 1953 outbreak are somewhat inflated by an intensive search for cases made in October and November. The outbreak was probably already under way when the school term began in September and continued through the autumn and the early part of the winter. There was a con

TABLE 1. *Presence of Staphylococcus aureus and of haemolytic streptococci in lesion swabs of cases of impetigo*

Number of lesions	School clinics 1953–54	School clinics 1954–55	School clinics 1955–57	Skin Hospital 1955–57
Total	190	183	141	95
containing *Staph. aureus*	158 (83 %)	155 (85 %)	114 (81 %)	92 (97 %)
Str. pyogenes	90 (47 %)	91 (50 %)	63 (45 %)	12 (13 %)
Haemolytic streptococci Group C	8	2	4	3
Haemolytic streptococci Group G	2	2	1	0
No *Staph. aureus* or haemolytic streptococci	6	11	10	

TABLE 2 *Phage-typing of Staphylococcus aureus strains from impetigo lesions*

Phage group	School clinics			Skin Hospital	
	1953–54	1954–55	1955–57	1955–57	Total
I.	4	4	18	6	30
II. Type 71	119 (75 %)	84 (54 %)	54 (47 %)	69 (75 %)	326 (63 %)
II. "71-like	2	6	3	3	14
II. Other patterns	7	13	9	1	30
III.	8	15	10	8	41
Not typable "71 like	1	12	2	0	15
Not typable Other	12	18	10	2	43
Unclassifiable	5	3	10	2	20
Total Type 71 & "71-like strains	122 (78 %)	102 (66 %)	59 (52 %)	72 (78 %)	355 (68 %)
Total	158	155	114	92	519

siderable falling-off of cases in February 1954 Small autumnal peaks occurred in 1954 and 1955 but thereafter the incidence was low and non-seasonal.

Bacteriology of Impetigo Lesions

The results of the bacteriological examination of one lesion from each of 609 patients are summarised in Table 1 It was convenient to divide the material from the school-clinics according to the school year which begins in September and since the number of cases examined in the two years 1955–56 and 1956–57 was rather small they will be considered together About 80 per cent of the swabs examined in each period were from cases in Salford, where a systematic study of the disease was being made No difference could be found between the results of the Salford swabs and those from neighbouring towns, and they will not be considered separately As might have been expected the proportion of notified cases examined bacteriologically was lowest when the disease was most common. Swabs were received from 20 % of the recorded cases in 1953–54 but the proportion had risen to 70 % by 1956–57 Despite the fall in the incidence of the disease during the period of the investigation, there was little change in the proportion of swabs yielding *Staph aureus* and *Str pyogenes* In each year four fifths of all lesions contained *Staph. aureus* Just under half contained only *Staph aureus* one-third yielded both *Staph. aureus* and *Str pyogenes* and a little over one-tenth *Str pyogenes* alone The findings in the series of hospital out-patients, examined during the 2 years in which the disease appeared to be relatively uncommon, and shown in the last column of Table 1 were entirely different Here 97 % of the lesions contained *Staph aureus* but *Str pyogenes* was present in only 1·% %. The results were, in fact very like those obtained by Barrow (3) from hospital out patient in Bradford in 1953–54

Table 2 gives the results of phage-typing the *Staph aureus* strains and shows the proportion belonging to Type 1 and to certain other groups. Type 71 was by far

TABLE 3 *Presence of Streptococcus pyogenes strains reacting with sera 3/13/B3264 5/11/12/27/44 & 8/25/Imp 19 in impetigo lesions*

Strains described as "mixed" reacted with 2 or more sera of each of the tw groups 3/13/B3264 & 5/11/12/27/44).

	School clinics			Skin Hospital 1955–57	Total
	1953–54	1954–55	1956–57		
Total *Str pyogenes*	80*	81	83	12	..54
3/13/B3264	33	35	32	2	103
5/11/12/27/44	31	38	11	3	84
Mixed"	0	2	6	1	4
8/25/Imp. 19	2	2	12	0	16
Other types	17	15	8	5	45

* 4 strains not typed.

the most commonly encountered type in all years and the rest of the strains included a great variety of different phage patterns none of which occurred frequently There was a steady fall in the proportion of Type 71 strains in the school-clinic series from 75 % in 1953–54 to 47 % in 1955–57 In the hospital out patient series, the percentage in 1955–57 was as high as it had been in the school clinics at the peak of the impetigo epidemic Table also shows that a number of strains in the rest of phage Group II, and of non-typable strains, with 71 like characters were also isolated these strains all gave sharp inhibition zones on *C d ptheriae* and were egg yolk negative If they are accepted as possible causes of impetigo the difference between the findings in 1953–54 and 1954–55 would be somewhat reduced

In Table 3 the results of typing strains of *St pyogenes* are shown. In both 1953–54 and 1954–55 the two impetigo types 3 13/B3264 and 5/11/1 /*7/44 together made up 70 % of all strains but in the last years there w a relative deficiency of 5/11/1 /*7 44 strains It is interesting to note that during this latter period a number of strains agglutinated by the sera 8/25/Imp 19 were isolated, as similar strains had made up nearly one third of a series from impetigo among troops examined by Dr K Crowley in 1941 (see Parker *et al.* (19). Four strains reacting with .. sera from each of the groups 3/13/B3264 and 5/11/1../*"/44 (recorded as mixed strains in Table 3) were also seen.

Some further indication of the importance of the various types can be obtained from the streptococcus type distribution surveys carried out twice a year by the Public Health Laboratory Service (Report 195" and further unpublished results) In 13 surveys there were streptococci from a total of 60 cases of impetigo 35 % had agglutination patterns in 3/13/B3*64 1 in 5/11/12/*7/44, and 5 % in 8/..5 The percentage among the 2660 streptococci from all sources were respectively 5 12, and 6 The excess of strains in the 3/13/B3264 group is therefore clear that in the 5/11/1 /*7/44 group is less clear and there is no excess in the 8/25 group

The serological reactions of the impetigo

TABLE 4. *The association of specific types of Staphylococcus aureus and of Streptococcus pyogenes in patients with impetigo*

	Number of cases with streptococci: types 3/13/B3264, 5/11/12/27/44 or mixed	other types	absent	Total
Cases with staphylococci				
Type 71 & 71-like	65	22	266	353
Other types	76	30	57	163
Absent	50	9	30	89
Total	191	61	353	605

streptococci have also been investigated in more detail. Using the methods for absorption of typing sera described by McLean (16) it has been found that most recently isolated impetigo strains in the agglutination group 5/11/12/27/44 are probably in fact Type 11. There is some indication that impetigo streptococci, whether in the 3/13/B3264 or the 5/11/12/27/44 group, may share a common antigen not found in strains from other lesions (Fraser, unpublished observations). These relationships will require further study, but they may well lend support to the idea that there are specific characters associated with the streptococci that cause impetigo.

The Association between Staphylococcal and Streptococcal Types in Impetigo Lesions

If impetigo may be either staphylococcal or streptococcal, and only certain strains of either organism can cause it, one might expect that the staphylococcal cases would yield the specific staphylococcus but that the streptococci found in them might be of any type, and conversely that the streptococcal cases might have a variety of types of staphylococci.

For Table 4 the specific impetigo staphylococci were taken as those of Type 71 and the 71-like Group II and untypable strains; impetigo streptococci were those with agglutination patterns 3/13/B3264 or 5/11/12/27/44 or mixtures of the two.

Of the 353 cases yielding the specific staphylococci, 87 (25%) yielded some streptococci whereas of the 252 cases not yielding impetigo staphylococci, 163 (66%) yielded streptococci. In both groups, however, some 76% of the streptococci belonged to the impetigo types. In the P.H.L.S. surveys 17% of all streptococci belonged to these types.

There were 191 cases with impetigo streptococci and 141 (74%) of these yielded staphylococci; there were 414 cases with no impetigo streptococci and 375 (91%) yielded staphylococci. In the two groups the proportion of the staphylococci in the impetigo types were 46% and 77% respectively. In a survey of staphylococci in lesions other than impetigo in out-patients (17) only 7% belonged to Type 71. Williams (23) found only [illegible] of Type 71 strains among staphylococci from septic lesions in hospital patients.

It seems then that when impetigo staphylococci were absent there was an increased frequency of streptococci, and conversely. But whether staphylococci were present or not about 6[illegible] of the

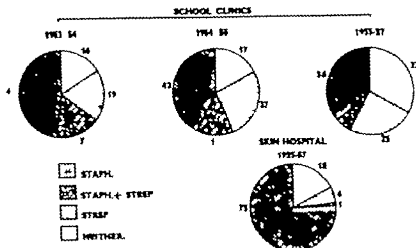


Fig ... Percentage of lesions from which impetigo streptococci and impetigo staphylococci were isolated alone or together

streptococci were of the specific impetigo types, as though perhaps these types were especially well adapted to life on the skin. In all cases a large proportion of the staphylococci belonged to Type 71 but the proportion was greater in lesions yielding no streptococci.

Fig. 2 shows the percentage of lesions associated with impetigo staphylococci and impetigo streptococci alone or together If impetigo is considered staphylococcal when it contains only *Staph aureus* Type 71 or a 71-like strain, streptococcal when it contains only *St pyogenes* with agglutination patterns in 3/13 B3*64 5/11/12/ *"/44 or in both, and mixed when it contains both staphylococci and streptococci of impetigo types then 43 .. of the school clinic cases were presumably staphylococ cal 4 % streptococcal and 12 mixed. The percentage of staphylococcal cases fell steadily as impetigo became less common (48 in 1953-54 30 .. in 1955-57) Most of the mixed cases occurred in the ea ly years and streptococcal cases were commoner in the later years than in 1953-54 The percentage of cases from which no specific organism was isolated was greatest when the incidence of the disease was lowest. In the series of hospital outpatients 5 % of the cases were presumably staphylococcal, 6 % streptococcal and 1 % mixed.

Staphylococcal Infections of Newborn Infants

Between September 1953 and August 1957 532 strains of *Staph aureus* from infant lesions were received for typing at the Manchester Public Health Laboratory They included representative strains from 34 outbreaks of sepsis and 13 sporadic infections in 22 hospitals in north-west England. N ttempt was made to select the material, which represents those incidents in which hospital pathologists considered that they needed the help of phage-typing to investi gat and control an outbreak. Staphylococci were also obtained from 1 small outbreak and 3 sporadic infections in infants born at home

Table 5 summarises the typing results on the strains from all sorts of lesions. As

TABLE 5 *Phage-typing of 532 Staphylococcus aureus strains from lesions of newborn infants*

Phage group		Skin vesicles, bullae or spots	Other skin lesions	Umbilical sepsis	Conjunctivitis	Deep suppuration
I.		92	3	39	49	[illegible]
II.	Type 71	67		0	7	1
	"71-like A	3	0	0	1	0
	"71 like B	9	1	0	2	0
	Other patterns	7	0	1	4	2
III		43	4	31	35	[illegible]
Not typable	"71-like	1	0	0	0	0
	Other	39	2	8	5	4
Unclassifiable		2	0	5	6	[illegible]
Total		263	12	84	129	44

is usual representatives of a great many phage patterns were found Only 14 % of all the strains, but 25 % of those from vesicles bullae and spots on the skin belonged to Type 71 Only 6 % of strains from other superficial situations, and 2 % from deep lesions were of this type Phage Group II strains other than Type 71 were as is usual in hospitals. It is to note that over half of them ere not Group II strains of the sort commonly found in boils and abscesses in general practice but were '71 like Those referred to as '71 like A were identical with the strains seen in impetigo lesions, and those described as '71 like B all had the phage-pattern 55/71 did not inhibit *C diphtheriae* but were egg yolk negative and penicillin resistant They were therefore identical with the DI negative variant which appears in practically all Type 71 cultures on storage in the laboratory (18) Such strains were also isolated in some outbreaks in close epidemiological association with ordinary Type 71 strains, and in one outbreak both forms were isolated from each of two patients on the same occasion

In Table 6 information is given about the 35 outbreaks and 17 sporadic cases from which these strains had been isolated. It shows the phage-typing results on all strains responsible for skin sepsis in each incident, strains obtained only from sticky eye or from trivial umbilical infections are excluded. In 26 outbreaks only one strain was involved but 24 different strains were isolated in the remaining 9 outbreaks. Although Type 71 was responsible for only a quarter of all the skin spots, it was one of the infecting types in 19/35 (54 %) of all outbreaks and was the only strain isolated in 14/26 (54 %) of outbreaks due to one type. Two '71-like strains also gave rise to outbreaks. The average size of the outbreaks due to Type 71 was rather small (usually 5 cases or less) In the last column of Table 6 are the 17 strains from single cases of skin sepsis 14 arising in hospital and 3 in babies born at home Ten were due to Type I including all three of the home deliveries. One of the latter appeared to have been contracted from an older child with impetigo

We obtained accounts of the clinical

TABLE 6 *Phage-typing of the strains of Staphylococcus aureus responsible for 35 outbreaks & 17 sporadic cases of skin sepsis in newborn infants*

Phage group		All strains	Outbreaks due to single strain	Sporadic cases
I.		14	5	0
II.	Type 71	19[a]	14[b]	10
	"71-like" A	1	1	0
	"71-like" B	1	1	0
	Other patterns	0	0	1
III.		12	4	0
Not typable	"71-like"	0	0	1
	Other	3	1	5
Unclassifiable		0	0	0
Total		50	26	17

[a] 3 with associated cases due to "71-like" B
[b] 2 with associated cases due to "71-like" B.

condition of many of the infants who had skin infections due to *Staph aureus* Type 71 The lesions varied greatly in severity but the characteristic appearance was of a superficial blister with little surrounding inflammatory reaction Sometimes only a few small vesicles were present, at others large confluent bullae Occasionally a generalised exfoliative dermatitis occurred. We have encountered four such cases which were diagnosed clinically as Von Ritter's disease Two of these formed part of a small outbreak consisting of two cases of generalised exfoliative dermatitis and one case of impetigo associated with a midwif in domiciliary practice The first infant died rapidly from an exfoliative dermatitis, from which *Staph. aureus* was grown in pure culture but was not typed A few days later a few clear blisters like scalds appeared on the arm of an older sister of the baby but no cultures were made The second baby in a different household in the same village was delivered by the same midwif about a fortnight later It suffered from a severe attack of exfoliative dermatitis but survived. *Staph aureus* Type 71 was isolated from the skin lesion of the second baby and from the nose of the midwife

Discussion

The information presented here lends some support to the view that the superficial vesicular or crusted non-scarring lesions diagnosed clinically as *impetigo contagiosa* include a staphylococcal disease due to *Staph aureus* Type 71 and a streptococcal disease associated with *St pyogenes* type 3/13/B3264 or 5/11/1 /47/44

The material examined consisted of single swabs from the lesions of impetigo cases seen by a number of medical officers, on first attendance at a school clinic or a hospital out patient department and included swabs collected at all stages of the disease One or more of the organisms thought to be associated with impetigo was isolated from 79 % of the cases The remaining 1 % may have included some in which the causative organism had dis-

appeared before the lesion was swabbed, some in which its presence was masked by secondary invasion by another strain of the same species, others in which the diagnosis was incorrect, and possibly also cases due to other strains of *Staph. aureus* or of *Str. pyogenes* the association of which with impetigo has not yet been recognised.

In the whole series, staphylococcal impetigo appeared to be about twice as common as streptococcal, and there were considerable differences in the relative proportions from year to year. A most unexpected finding, however, was the great difference between the flora of lesions swabbed in school clinics and the out-patient department of the skin hospital. Although staphylococcal cases were at all times more common than streptococcal cases in the school clinics, the difference was not great, except during the impetigo epidemic in 1953, and the two specific types of *Str. pyogenes* were at no time isolated from less than 30 % of the lesions. In the hospital out-patient series, which was examined at a time of low incidence of impetigo in the general population, *Staph. aureus* Type 71 was isolated from nearly three-quarters of the cases, and *Str. pyogenes* was seldom seen. A number of possible explanations of this were considered. It might be thought that *Str. pyogenes* was present frequently in early untreated cases seen in the school clinics, and that secondary infection with *Staph. aureus* either naturally or in consequence of penicillin treatment, had caused the disappearance of the streptococci before the cases were referred to the skin hospital. This did not seem to be so, since there was no difference in the frequency with which *Staph. aureus* Type 71 was isolated from those skin hospital cases seen within 7 days of onset and from those seen later. The possibility that the cases which yielded *Str. pyogenes* were given some other diagnostic label by the physicians at the skin hospital was also considered. A sample of other superficial lesions seen in the same out-patient clinics was examined, but very few strains of the specific types of *Str. pyogenes* were isolated. We were therefore forced to the conclusion that the cases which commonly yielded *Str. pyogenes* did not often reach the hospital out-patient department. This further strengthened our conviction that the school clinic cases included two separate diseases, one of which was not severe enough to be referred to hospital.

Opinion is divided among dermatologists as to whether a clinical distinction between staphylococcal and streptococcal impetigo can be made. It is notable that those who have made the distinction with most confidence have had experience of severe outbreaks of bullous impetigo in which only *Staph. aureus* can be isolated from most of the lesions (6, 8, 10, 4). Such conditions appear to occur frequently in countries with a hot dry summer (8), but the careful clinical observations of Friedberg (12) suggest that the distinction can be made on sporadic cases occurring in temperate climates. It is possible that the clinical diagnosis of staphylococcal impetigo is easily made only when the lesions are severe enough for cases to be seen while the vesicles or bullae are unruptured, and this seems to have been the case during the earlier part of the Salford epidemic in 1953. At other times the initial vesicle appears often to have been

small, and to have ruptured early By the time the cases were seen, either in the school clinics or at the skin hospital, the lesion had become crusted and was not very characteristic

Our findings go some way towards explaining the nature of the mixed infections with *Staph aureus* and *St pyogenes* which made up about one-third of the school clinic cases. The typing results suggested that most of them were secondary staphylococcal infections of streptococcal lesions. However the relative frequency with which *Staph aureus* Type 71 and a specific streptococcus were found together in the same lesion remains unexplained.

The part played by *Staph. aureus* Type 71 in the skin infections of newborn infants has been obscured by the fact that it seldom gives rise to large epidemics in hospital, possibly because it seldom becomes widely disseminated among the contacts of cases In the years 1953–57 it gave rise frequently to single cases and small epidemics in north west England. It appears to have been rarer in the south of England than in the north during these years, but to have given rise to epidemics of the same general sort in both areas Williams (25) found only 20 Type 71 strains among 403 independent strains from maternity hospitals in southern England in the years 1954–57 and the average number of cases in seven outbreaks was 4 3

The lesion from which it is isolated consist characteristically of an intraepidermal vesicle with little inflammatory reaction in the deeper tissues in striking contrast to the more boil like lesion often with underlying cellulitis caused by other strains including Type 80 Severe lesion due to Type 71 sometimes occur and involve large areas of skin, but the lesion is confined to the epidermis

Summary

Six hundred and nine impetigo lesions were examined bacteriologically and the presence in them of certain types of *Staph. aureus* and of *Str pyogenes* thought to be specifically associated with impetigo was determined.

On the basis of the bacteriological findings, 47 % of the cases were considered to be staphylococcal, 21 % streptococcal and 11 % mixed. No specific pathogen was isolated from 21 %.

Among children seen in school minor ailment clinics, staphylococcal cases predominated when impetigo was prevalent but at all times a fifth or more of the cases were streptococcal Cases referred to the out patient department of a skin hospital were however nearly all staphylococcal.

It is probable that impetigo includes a more severe often bullous skin infection due to *Staph aureus* Type 71 and a less severe condition associated with certain types of *St pyogenes*

Staph aureus Type 71 was also responsible for a number of outbreaks of skin infection among newborn infants in hospital. Cases were of all degrees of severity from a mild vesicular eruption to a fully developed *pemphigus neonatorum* and a few cases of generalised exfoliative dermatitis were seen.

Similar cases due to Type 71 were occasionally seen in domiciliary practice infected either from the midwife or from an older child with staphylococcal impetigo

The characteristic skin lesion caused by *Staph aureus* Type 71 is an intraepidermal vesicle or bulla with little inflammatory reaction in the deeper tissues.

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References

1 ALMQUIST E.: Pemphigus neonatorum, bakteriologisch und epidemiologisch beleuchtet. *Z Hyg I fektkr 10* 253 1891
2 ANDERSON B. S. and WILLIAMS, R. E. O Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology *J Clin Path, 9* 94 1956.
3. BARROW G. I Clinical and bacteriological aspects of impetigo contagiosa. *J Hyg (Lond)*, *53* 495, 1955.
4 BURN J L.: In *Ann Rep M O.H City of Salford* 1953. 1954.
5. CLEGG, M. T and WHERRY W B The etiology of pemphigus contagiosus in the tropics. *J Infect Dis 3* 165, 1906.
6 DOHI, K. and DOHI S.: Zur Klinik und Ätiologie der Impetigo contagiosa. *Arch Derm Syph* (*Wien*) *111* 629 1912.
DUNN R. W On porrigo. *Brit Med J II* 576, 1862.
ENGMAN M. F Impetigo contagiosa bullosa and its bacteriology *J Cutan Dis, 19* 180 1901
9 EPSTEIN S.: Beitrag zur staphylogenen Impetigo contagiosa. *Derm Z, 70* 3.8, 1935.
10. — Staphylococcic impetigo contagiosa. *Arch Derm Syph* (*Chicago*), *42* 840 1940.
11 FOX, W T On impetigo contagiosa, or porrigo. *Brit Med J I* 467 1864
12 FRIEDBERG, R. Bacteriological studies on impetigo, especially the streptogenic form. *Acta Dermatovener (Stockh)*, *23* 297 1942.
13. GILLESPIE, W A., POPE, ROSEMARY C and SIMPSON K.: Pemphigus neonatorum caused by Staphylococcus aureus, Type 71 *Brit Med J I* 1044 1957
14. GRIFFITH, F The serological classification of Streptococcus pyogenes. *J Hyg* (*Lond*) *34* 542, 1934.
15. MATZEN UER, R. Zur Frage der Identität des Pemphigus neonatorum und der Impetigo contagiosa. *Wien Klin Wschr 13*. 1077 1900.
16. McLEAN SIBELY J.: Identification of strains of Streptococcus pyogenes of types, 5, 11 12, 27 and 44 by the precipitin test for the T antigen. *J Gen Microbiol*, *9* 110, 1953.
17 PARKER, M. T Some cultural characteristics of Staphylococcus aureus strains from superficial skin infections. *J Hyg* (*Lond*), *56* 238, 1958.
18. PARKER, M. T and SIMMONS, L. E.: The inhibition of Corynebacterium diphtheriae and other Gram-positive organisms by Staphylococcus aureus. *J Gen Microbiol*, *21* 457 1959
19. PARKER, M. T TOMLINSON A J H and WILLIAMS, R. E. O.: Impetigo contagiosa. The association of certain types of Staphylococcus aureus and of Streptococcus pyogenes with superficial skin infections. *J Hyg* (*Lond*), *53* 458, 1955.
20. REPORT: Serotypes of Streptococcus pyogenes their relative prevalence in England and Wales 1952 1956. *Mon Bull Min Hlth Lab Serv 16* 163, 1957
21 — International Committee on Phage Typing of Staphylococci. Minutes of 2nd Meeting *Int Bull Bact Nomencl Taxon*, *9* 113, 1959
22. SPITTLEHOUSE, K. F Phage-types of Staphylococcus pyogenes isolated from impetigo and sycosis barbae *Lancet*, *II* 378, 1955.
23. SWIFT H. F WILSON A. T and LANCEFIELD REBECCA. C Typing group A haemolytic streptococci by M precipitation reactions in capillary pipettes. *J Exp Med 78* 127 1943.
24 T CH D P The bacteriology of impetigo contagiosa. *Brit J Derm*, *40* 112, 1928.
25. WILLIAMS, R. F O Epidemic staphylococci. *Lancet I* 190, 1959

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Sensitivity of Listeria Monocytogenes *in vitro* to Different Antibiotics and Chemotherapeutics

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During the period Jan. 1 1948 to April 30 1960 177 *Listeria monocytogenes* (*L.m.*) strains have been isolated and collected in our Institute. In view of the increasing interest in listeriosis in Sweden we have studied a number of these strains with respect to their sensitivity *in vitro* to various antibiotics and chemotherapeutics. The classification of antibacterial substances according to origin is not considered necessary in modern medical terminology.

As has been pointed out *inter alia* by Ericsson & Svartz-Malmberg (3) the antibacterial spectra to antibiotics are not constant but vary from one time and one environment to another. For this reason it is of interest to study a large number of bacterial strains of the same species in order to be able to note any variations that occur in their degree of sensitivity to different antibiotics.

Survey of Literature

For general information on animal listeriosis in Sweden the reader is referred to Nilsson & Karlsson (6). So far as we have been able to discover from the available literature, apart from *Linzenmeier & Seeliger*'s survey (5) of the sensitivity to antibiotics of 25 L.m. strains, no large number of these strains has been studied at any one time by the same method. On the other hand, there are numerous reports on the sensitivity of single strains to different antibiotics. Seeliger (7) summarizes the results to the effect that the tetracyclines (chlortetracycline, oxytetracycline, tetracycline) show the greatest antibiotic effect, followed by the sulphonamide compounds, the latter being most active in combination with penicillin. Resistance quickly develops against streptomycin. The use of chloramphenicol and erythromycin appears to give promising results.

Cronin & Moran (1) have reported that 9 L.m. strains investigated are sensitive to low concentrations of nitrofuran compounds *in vitro*.

Material and Method

Of 177 L.m. strains isolated at the Swedish State Veterinary Medical Institute from 13 animal species, a study has been made of 56 strains (Table 1).

After the primary isolation the strains were passed at regular intervals and kept suspended in physiological salt solution at 20 C.

Twenty four of the strains investigated

TABLE 1 Listeria monocytogenes *isolated at the State Veterinary Medical Institute 1/1/1948–30/4/1960*

Animal species	No. of isolations	No. of strains tested
Poultry	124	54
Chinchilla	20	15
Hare	9	
Cattle	5	1
Sheep	3	3
Rabbit	3	1
Roe deer	3	1
Fox	2	—
Pig		1
Fallow deer	1	1
Turkey	1	—
Cat	1	—
Partridge	1	1
Vole	1	—
Wildduck	1	1
Total	177	86

have been earlier serotyped by Dr H. liger in Bonn, all being found to belong Serotype I.

The sensitivity tests were performed by an agar diffusion technique described by Ericsson *et al.* (2, 3, 4). The strains were denoted as "sensitive", "fairly sensitive", "slightly sensitive" and "resistant". These designations correspond to Groups I, II, III and IV. The clinical evaluations of these four groups, as proposed by Ericsson *et al.*, are:

I "Sensitive" (likely to yield to therapy in general infection, ordinary dosage).

II "Fairly sensitive" (likely to yield to therapy in general infection; high dosage).

III "Slightly sensitive" (likely to yield to therapy in organ in which the agent may be concentrated, e.g. certain urinary infections).

IV "Resistant" (therapeutic effect unlikely).

This was the basis of evaluation for nine of the antibiotics tested. The *polymyxin* however, come into a different category. Owing to their high molecular weight and other physicochemical properties they have a slow rate of diffusion. Ericsson & Svartz-Malmberg (3) therefore advise against using the *quantitative* variant of the disc method for this antibiotic.

Neomycin and *bacitracin* are characterised by their toxicity and with oral therapy effective concentrations are not possible except in the intestinal contents. Parenteral application (e.g. in urinary infections) often involves a risk and should be reserved for treatment when especially sensitive strains are present. For these reasons Group I was excluded when evaluating the results with these agents.

Aerobacin cannot be concentrated in the urine. For this antibiotic, therefore Group II should be directly followed by Group IV.

Nitrofurantoin, on the other hand, attains satisfactory therapeutic concentrations only in the urinary tract. Quantitative determination of nitrofurantoin is therefore superfluous for bacteria of Groups I and II. But for the clinician all strains which are inhibited by a Group III nitrofurantoin concentration are fully suited for therapy.

Results and Discussion

The distribution of sensitivity within the group of L.m. strains investigated, expressed in per cent, is shown in Table 2. As is seen, the tetracyclines (chlortetracycline, oxytetracycline, tetracycline), erythromycin and streptomycin are most effective against L.m. *in vitro*.

About 40 per cent of the investigated strains show resistance to sulphonamide, a higher figure than reported by Linzenmeier & Seeliger (5). It is also apparent from Figs. 1 and 2 that there is no essential difference in resistance referable to the species of animal from which the strains emanate.

As regards the tetracyclines, the present

TABLE 2. *Antibiotic sensitivity of Listeria strains expressed in per cent*

Group	I	II	III	IV
Characterization	"Sensitive"	"Fairly sensitive"	"Slightly sensitive	"Resistant
Sulfaisodimidine	20.9	20.9	17.5	40.7
Penicillin	4.7	74.4	20.9	0
Erytromycin	63.9	34.9	1.2	0
Oleandomycin	19.8	70.9	9.3	0
Novobiocin	4.7	52.3	See text	43.0
Streptomycin	94.1	4.7	1.2	0
Chlortetracycline	81.4	18.6	0	0
Oxytetracycline	74.4	25.6	0	0
Tetracycline	79.1	20.9	0	0
Chloramphenicol	12.8	83.7	3.5	0
Bacitracin	See text	0	1.2	98.8
Neomycin	See text	98.8	0	1.2
Polymyxin	0	0	0	100
Nitrofurantoin	See text	See text	76.7	23.3



Fig. 1. Resistant strains of *Listeria monocytogenes* expressed as percentage of total number of strains isolated from various species. I, chickens (54 strains); II, chinchilla (15 strains); III, other species (17 strains).

results are in close accordance with Seeliger's data (7) on the effect of these antibiotics *in vitro* and *in vivo*. Practical experience shows treatment with tetracyclines to be of great value having reduced the mortality even among severe cases of listeriosis. This only on condition, however of early administration of therapy, maximum dosage and adequate period of treatment with respect to the risk of recrudescence.

About 1 per cent of the L.m. strains investigated were "fairly sensitive" to tetracyclines. This emphasizes the im

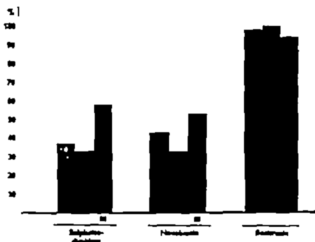


Fig. —. Resistant strains of *Listeria monocytogenes* expressed as percentage of total number of strains isolated from various species. I, chickens (54 strains); II, chinchilla (15 strains); III, other species (17 strains).

portance of deciding on the most beneficial therapy in every case by means of sensitivity tests *in vitro*.

Summary

The sensitivity of 14 antibiotics and chemotherapeutics to 86 strains of *Listeria monocytogenes* isolated from 15 animal species was studied by means of an agar diffusion technique. The tetracyclines (chlortetracycline, oxytetracycline, tetracycline), erythromycin and streptomycin proved to be clearly superior to other antibiotics tested *in vitro*.

No difference in resistance was found referable to the species of animal from which the strains emanated.

References

1. Cro... M. T. I. and M... A. B.: A note on the in vitro sensitivity ... of Listeria monocytogenes. Vet. Med. ... 3: 1 ... 19..
2. Eri...so.. H. F... ... I. T. ... G. and W... G.: Best... bakterier ... Svensk. Lak. tid. ... 1953.
3. Eri...s... H. and S... M. ... G.: Determination of bacterial sensitivity in vitro and ... ... Antibiot. Chemother. (Basel) 4: 41 ... 1957.
4. Eri...s... H. R.: ... use of antibiotics in hospitals. Scand. ... J. Cl... Lab. ... 12 Suppl. 50 1960.
5. ... Seeliger, O. and ...: ... in vitro Empfindlichkeit von Listeria monocytogenes (Pirie) gegen Sulfonamide und Antibiotica. Zbl. Bakt. (Orig.) 160: 313-33.., 1953/54.
6. Nyström, A. and Karlsson, K.-A.: Listeria monocytogenes isolations from animals in Sweden during 1948-195.. Nord. Vet. Med. 11: 303-315, 195..
7. ... H.: Listeriose. Johann Ambrosius Barth Verlag, Leipzig 1958.

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Nocturnal Enuresis in Twins[1]

II. Urethro-Cystographic Examinations

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Introduction

The aim of the present investigation is to study by means of micturition urethrocystography whether any association between nocturnal enuresis and changes in the lower urinary tract can be shown, and if so to consider the nature of the relationship. For this purpose a series of enuretic twin children and their partners were selected for investigation. The effective bladder capacity will also be examined.

In the present study nocturnal enuresis is defined as repeated involuntary micturition during sleep after the fourth year of life. The definition does not include cases of urinary incontinence caused by any gross organic lesion in the urinary tract, the nervous system or by systemic diseases, e.g. ectopic ureters, lesions of the spinal cord, diabetes insipidus. When the enuretic child had been dry for about one year or more and had not begun to wet again, the enuresis is referred to as previous enuresis.

Opinions differ as to the causal significance of urinary tract disorders in enuresis. Several investigators who have conducted special examinations of the lower urinary tract in enuretics state that they have found organic changes of different kinds in about half the cases—for references see *inter alia* Kjellberg, Ericsson & Rudhe (6) and Hallgren (4).

Kjellberg *et al.* examined consecutive series of 593 enuretic children by urethrocystography during micturition, using a specially devised method. They could not show any specific organic changes, but found changes described as pathological in 21 per cent of the children. In 80 per cent of these cases, pathological conditions were also observed by endoscopy, usually in the form of trigono-urethritis. The changes were more frequent in the group in which nocturnal enuresis was complicated with diurnal enuresis. It was particularly emphasized that no obvious cause and effect relationship existed between such findings and the occurrence of enuresis.

Giertz & Lindblom (2), examining urethrocystographically 11 adult patients with nocturnal enuresis, stressed that the posterior urethra, especially at the level of the external sphincter, was particularly wide during micturition.

Some authors have stressed that a proportion of the children with enuresis have a

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TABLE 1 *Distribution of the material by age zygosity and sex*

Age (years)	MZ pairs			DZ pairs			Total		
	□□	○○	Total	□□	○○	Total	□□	○○	Total
7	1	—	1	2	—	2	3	—	3
8	2	1	3	9	2	11	11	3	14
9	1	3	4	7	—	7	8	3	11
10		1	3	4	1	5	6	2	8
11	—	—	—	1	1	2	1	1	
12	—	—	—	1	1	2	1	1	2
Total	6	5	11	24	5	29	30	10	40

lower effective bladder capacity than other children—*cf inter alia* McLellan (7), Stalker & Band (10), Nash (8) Vulliamy (12) There appear however to be no studies with adequate statistical comparisons between groups of enuretics and controls. Poulton & Hinden (9) reported a functional bladder capacity below the norm in 12 out f 200 (6 per cent) children with enuresis. The normal values were based on the findings in 18 "normal children An imperative need to empty the when its capacity was reached was reported in a proportion of cases by *cf* McLellan (7) and Stockwell & Smith (11) On the other hand Kjellberg Ericsson & Rudhe (6) making cystometric investigations did not find any specific pathological features

Material

The present cases were selected from a representative series of twins consisting f 911 pairs—cf Hallgren (5) The present material comprises all same-sexed twin pairs in which at least one of the partners suffered from persistent nocturnal enuresis, viz. 40 pairs including 49 affected individuals, the propositi. The distribution f the cases by age zygosity and sex when examined appears in Table 1

Two pairs originally selected had to be excluded, because the X ray f lms n, respectively one and both partners were not f good enough quality to perm t a detailed scrutin

A third pair was luded because the twin who wetted had a hypospadia ah pronounced stenosis of the external meatus He did not cease to wet after meatotomy but for practical reasons a new roentgenological examination of both twins cold not be arranged.

Finally 7 pairs (13 per cent) had to be excluded because, for various reasons, the mother was not willing to cooperate in the urethro-cystographic examination. The comprehensive case histories that were available do not indicat that these pairs represent a selection in respect of any relevant factor

When making intra pair comparisons, those 9 pairs in which both partners were propositi, were excluded. Such comparisons are thus made on the basis of 31 pairs. In 4 of these pairs the twin partner of the propositus had a previous nocturnal enuresis.

The enuresis was primary in all but ra cases, i.e the child had no dry period before the age of 4 lasting continuously for approximately one year or more A further three cases had a dry period after the age of 4 In 12 cases nocturnal enuresis was complicated with diurnal enuresis. The majority of the propositi 43 out of 49 (88 per cent) had wetted once a night or more during long period The other propositi had etted on an a erage once or several times a week. At the time of examination 18 cases wetted almost once a night or more

The advantages of investigating a t n series are obvious. Monozygotic (MZ) twins, being genetically identical, offer unique portun ties of studying the effect of en

genetic factors. Unfortunately as regards nocturnal enuresis the degree of concordance is so high that it is difficult to obtain a sufficiently large series of discordant monozygotic twin pairs for statistical analysis. However dizygotic (DZ) twins also are well adapted to intra-pair comparisons, since certain fundamental non-genetic factors can be kept under control, e.g. age and environmental factors both within and outside the family

Methods

The subjects were examined by *urethrocystography during micturition* by the methods described by Kjellberg *et al* (6) Both twins in a pair were examined on the same day 30 of them at the Roentgenological Department, Paediatric Clinic, Karolinska Sjukhuset, Stockholm, and 10 at the Roentgenological Department, Children Hospital, Gothenburg The former subjects, propositi as well as partners, were admitted t the paediatric clinic as in patients n connection with the examinations the subjects from Gothenburg were examined as out patients. All films were finally scrutinized by the same investigator (U.R.), who did not know which of the twin partners was enuretic

The *bladder capacity* was tested only on the subjects investigated t the Karolinska Sjukhuset. It was determined as the volume of contrast medium (non-irritant barium suspension) that the children could tolerate, when the medium was injected in the bladder This means the effective bladder capacity during an environmental strain which is equal for both partners

Cystometric investigations may give additional information Such a procedure was, however difficult to carry out for practical reasons, as the examinations had t be made as part of the routine examinations of the roentgenological department Since the effec t bladder capacity was considered to be of most interest for the present stud w refrained from cystometry

The determination of zygosity was made on the basis of anthropological criteria, blood groups and test abilit —cf H llgren (5).

In the statistical analysis[1] the following methods were employed. (For further details cf e.g Dixon & Massey (1).)

When testing the hypothesis that a number of samples are taken from a homogeneous population, and when testing the agreement between an bserved and an expected distribution, we used the χ test Where the number of subjects in any of the groups to be compared is less than 10, we have refrained from making statistical analyses.

The standard error of the mean f the differences between paired observations, $\varepsilon_{\bar{d}}$, was obtained with the formula

$$\varepsilon_{\bar{d}} = \frac{\sigma}{\sqrt{n}}$$

where σ is the standard deviation, and n is the number f pairs of observations.

The standard deviation, σ was calculated from the formula

$$\sigma = \sqrt{\frac{\Sigma(d-\bar{d})^2}{n-1}}$$

where $(d-\bar{d})$ is the deviation of a difference from the mean of the differences, and n is the number of pairs of bservations.

When making intra-pair comparisons with regard to the occurrence of a certain quality a sign test was used—cf Dixon & Massey (1957; Table 10a, p. 417). Only those pairs were considered in which there was an intra-pair difference in respect of the quality

A difference is considered

significant	if $0.01 < p < 0.05$
significant	if $0.001 < p < 0.01$
significant	if p 0.001

Unfortunately the number of monozygotic twins was too small for separat st tistical analyses. Therefore monozygotic and dizygotic twins were considered together in the calculations. A separat analyses f the individual monozygotic pairs was also made

[1] Mr Staffan Ekblom, B.Sc., of the St tistical Research Group of the University of Stockholm, rendered us aluable help in the statistical treatment of the material.

TABLE 2 *Urethro-cystographic deviations in the lower urinary tract in twins with and without persistent nocturnal enuresis distribution by zygosity and sex*

	Propositi									Partner								
	MZ			DZ			Total			MZ			DZ			Total		
Deviation	□	O	Total	□	O	Total	□	O	T tal	□	O	Total	□	O	Total	□	O	T
Deficient relaxation of external sphincter	—	4	4	1	2	3	1	6	7	—	1	1	—	—	—	—	1	
Deficient widening of bladder neck	—	—	—	2	—	2	2	—	2	—	—	—	2	—	2	2	—	
Transverse f ld in posterior urethra	—	—	—		—	2	2	—	2	—	—	—	—	—	—	—	—	
Minimal reflux to ureter	1	—	1	1	—	1	2	—	2	—	1	1	1	—	1	1	1	
Total: With deviation	1	4	5	6		8	7	6	13	—	1	1	3	—	3	3	1	
Without deviation	7	4	11	"0	5	25	27	9	36	4	1	5	19	3	22	23	4	r
Total	8	8	16	"6	7	33	34	15	49	4	2	6	22	3	.5	26	4	1

Results

Urethro-cystographic examinations

Severely pathological changes were not found in any subject In the urethro-cystographic examinations, interest was focused on (1) deviations of a functional anatomical nature disclosed during micturition, (2) width of urethra particularly the posterior part (3) effective capacity of the bladder

The observed deviations consisted of a) deficient relaxation of the external sphincter during micturition with some dilatation of the urethra above this area b) deficient widening of the bladder neck c) the presence of a non-obstructive transverse fold in the posterior urethra at the level of the verumontanum d) ureteral reflux (only intramurally of the bladder)

The occurrence of the *different deviations* is shown in Table The frequency of deviations is approximately twice as high in the group of cases with persistent nocturnal enuresis *i.e* the propositi, 13 out of 40 ("7 per cent) than in the group of their partners, 4 out of 31 (13 per cent). The difference is however not statistically significant.

There is no significant sex difference in respect of the frequency of deviations in the group of propositi the figures being 7 out of 34 and 6 out of 15 respectively in that of partners 3 out of "6 and one out of 5. The number of observations is, however small.

In the frequency of deviations there is fairly good agreement between the group of MZ twins and the group of DZ twins

Pathological changes" are reported to be more frequent when nocturnal enuresis is complicated with diurnal enuresis. A comparison was therefore also made between the group of cases with persistent nocturnal and diurnal enuresis and the group of cases with persistent nocturnal enuresis only No significant difference between the groups emerged, the frequencies being 5 out of 1 and 8 out of 3" respectively Again, however the number of observations is small.

TABLE 3 *Intra pair differences between twins with persistent nocturnal enuresis and their partners in deviations of the lower urinary tract*

Propositus	Partner	MZ □	MZ ○	MZ Total	DZ □	DZ ○	DZ Total	Total □	Total ○	Total Total
Deviation	Deviation	—	1	1	2	—	2	2	1	3
Deviation	No deviation	1	—	1	2	1	3	3	1	4
No deviation	Deviation	—	—	—	1	—	1	1	—	1
No deviation	No deviation	3	1	4	17	2	19	20	3	23
Total		4	2	6	22	3	25	26	5	31

In order to make use of the advantage of the series being a twin sample an intra pair comparison was made Since the number of observations is comparatively small, the deviations were registered without regard to type of change It can be seen from Table 3 that in 5 pairs one of the twins had a deviation, the other not. In 4 out of these 5 cases the twin showing deviation was the propositus. The number of observations is too small to permit a statistical analysis.

The monozygotic twins were discordant in respect of deviations in three instances.

In two cases one of them being propositus, the deviation in question was ureteral reflux. One of the cases with reflux voided very frequently during the day the other was suspected of having had an infection of the lower urinary tract at the age of two. No other somatic factors of possible importance were found. One of the cases was considered to be emotionally somewhat less stable than his partner the other to be more stable than his partner but the differences were small. None of them had nervous problems.

In the third case propositus, the deviation in question was deficient relaxation of the external sphincter during micturition. This girl, as well as her twin sister who also had persistent nocturnal enuresis, wetted very frequently during the day No somatic factors of possible importance were found. The girl with deficient relaxation was considered to be somewhat less stable than her partner but had no nervous problems.

Width of urethra during micturition

By making intra-pair comparisons we could avoid the subjective decision of what is and what is not pathological in respect of the width of the urethra, i.e. its posterior portion and particularly at the level of external sphincter during micturition

It appears from Table 4 that an intra pair difference in the width of the posterior urethra was considered to exist in 10 instances, in 6 of which the propositus had the greater width. The distribution agrees rather well with that to be expected if there were no relationship between the calibre of the posterior urethra during micturition and nocturnal enuresis.

In 5 monozygotic pairs there was an intra-pair difference in the width of the posterior urethra during micturition; in three of these pairs both twins were propositi; in the two other pairs the twin who did not wet had the wider urethra during micturition

Disease of the urinary tract viz. a cystopyelitis at the age of 7 (two years before the present examination), occurred in one case who had greater width than her twin sister Diurnal frequency of micturition and urgency occurred in two pairs, both partners in each pair having the same micturition

TABLE 4 *Intra pair differences between twins with persistent nocturnal enuresis and their partners in the width of the posterior urethra*

Width of posterior urethra	MZ			DZ			Total		
	□	○	Total	□	○	Total	□	○	Total
Wider in propositus	—	—	—	6	—	6	6	—	6
Wider in partner	2	—	2	1	1	2	3	1	4
No difference	2	2	4	15	2	17	17	4	21
Total	4	2	6	22	3	25	26	5	31

TABLE 5 *Intra pair differences between twins with persistent nocturnal enuresis and their partners in the width of the urethra at the level of the external sphincter*

Width of urethra	MZ			DZ			Total		
	□	○	Total	□	○	Total	□	○	Total
Wider in propositus		—	2	4	1	5	6	1	7
Wider in partner	—	—	—	10	1	11	10	1	11
No difference	2	2	4	8	1	9	10	3	13
Total	4	2	6	22	3	.5	26	5	31

TABLE 6 *Mean width of urethra and intra pair differences in the width between twins with persistent nocturnal enuresis and their partners.*

Twin pa		Number of pairs of observations	Mean width of urethra		Mean of differences
			Propositus	Partner	
	□	4	6.23	7 12	—
MZ	○		4.50	4.25	—
□	○	6	5.67	6.17	—
	□	22	6.86	6 16	—
DZ	○	3	6.83	7 1	—
□	○	25	6.86	6.28	—
	□	26	6.77	6.31	—
Total	○	5	5.90	6.00	—
□	○	31	6.63	6.26	0.371 ± 0.231

symptoms [ ]pproximat l the same in tensity

An intra pair difference in the width of the urethra at the level of the external sphincter wa found in 18 instances In 7 of which the propositus had the greater width—*cf* Table 5 The distribution does not differ significantly from that to be expected if there were no relationship between nocturnal enuresis and width of urethra.

A lifference in width is considered to exist when it amou ts to 1 mm or more as measured in the films

TABLE 7 *Intra pair differences between twins with persistent nocturnal enuresis and their partners in bladder capacity*

Bladder capacity	MZ □	MZ ○	MZ Total	DZ □	DZ ○	DZ Total	Total □	Total ○	Total Total
Smaller in propositus	1	1	2	8	1	9	9	2	11
Smaller in partner	—	—	—	5	—	5	5	—	5
No difference	2	1	3	5	—	5	7	1	8
Total	3	2	5	18	1	19	1	3	24

TABLE 8. *Mean bladder capacity and intra-pair differences in the capacity between twins with persistent nocturnal enuresis and their partners*

Twin pair	Number of pairs of observations	Mean capacity Propositus	Mean capacity Partner	Mean of differences
MZ □	3	200	217	—
MZ ○	2	145	170	—
MZ □+○	5	178	196	—
DZ □	18	213	219	—
DZ ○	1	150	200	—
DZ □+○	19	210	218	—
Total □	21	211	218	—
Total ○	3	147	180	—
Total □+○	24	203	212.5	10.4 ± 8.2

In respect of the width at the level of the external sphincter there is a rather good agreement between the group of twins with persistent nocturnal enuresis and that of others — *cf* Table 6

Bladder capacity

The intra pair comparison in respect of the effective bladder capacity appears in Table 7 The bladder capacity was smaller in the propositus in 11 instances and in the partner in 5.

The observed distribution does not differ significantly from that to be expected if there were no association between nocturnal enuresis and bladder capacity

A difference in capacity is considered to exist when it amounts to 50 ml or more

There is no significant difference in respect of the mean of differences between the group of twins with persistent nocturnal enuresis and that of others—*cf* Table 8. The mean capacity in the two groups agrees fairly well.

There was a difference (between 50–90 ml) in the bladder capacity in 4 monozygotic pairs, in two of which both partners having persistent nocturnal enuresis. In none of the cases was there any noticeable difference between the pairs in respect of emotional stability or micturition symptoms. Nor was there in any case information on diseases of the lower urinary tract.

TABLE 9 *Intra-pair differences between twins with persistent nocturnal enuresis and their partners in the occurrence of spina bifida occulta*

Propositus	Partner	MZ □	MZ ○	MZ Total	DZ □	DZ ○	DZ Total	Total □	Total ○	Total
Spina bifida	Spina bifida	1	—	1		—		3	—	3
Spina bifida	No spina bifida	1	—	1	3	—	3	4	—	4
No spina bifida	Spina bifida	—	—	—	4	1	5	4	1	5
No spina bifida	No spina bifida	2	2	4	13	2	15	15	4	19
Total		4	2	6	22	3	25	[illegible]	5	31

In the calculations certain possible *biases* in the interpretation of the urethrocystographic findings were considered.

A priori, the observed deviations in the lower urinary tract as well as the width of the urethra during micturition and bladder capacity may be connected with previous or present lesions in lower urinary tract—in the present series only infections occurred, *e.g.* cystopyelitis—with certain micturition symptoms, *viz.* diurnal frequency of micturition (*pollakisuria*), urgency or with nervous tension during the examination. The possible influence on the results of the calculations in respect of enuresis of these biases was tested in the following way. The frequency of deviations found on urethro-cystography, the frequencies of cases with wider urethra and smaller bladder capacity than the partner in the respect ve groups of children with lower urinary tract infections, psychiatric problems (problem children ; *cf.* Hallgren (3)) and micturition symptoms were compared with the corresponding frequencies in the groups of children without urinary tract infections, psychiatric problems and micturition symptoms respectively. It emerged from the calculations that the above frequencies agreed rather well in the groups that were compared. Nor were there any significant differences in the frequencies between the group of twins which were considered by their mothers to be emotionally less stable than the respective co-twins and the group of the emotionally more stable partners. Hence the occurrence of lesions in the lower urinary tract, micturition symptoms and psychiatric symptoms could be disregarded in the calculations with regard to enuresis.

Furthermore, if there is any association between enuresis, on the one hand, and deviations in the lower urinary tract, width of the urethra or bladder capacity on the other hand, it is possible that cases with previous enuresis may disclose persistent pathological conditions. If such were the case this would affect the result of the comparisons. However the result is not changed if the 6 cases with previous nocturnal enuresis are excluded from the calculations. Therefore in the calculations these 6 cases have been included in the group of non-enuretics.

Spina bifida occulta

The roentgenological examination also permitted a registration of the occurrence of spina bifida occulta in the lower lumbar and the sacral regions. This anomaly located in L_5 or/and S_1 and S_2 was found in 9 out of 40 propositi and in 8 out of 31 of their partners. The difference is not significant. The intra-pair comparison shows that in 9 pairs only one of the twins had spina bifida — *cf.* Table 9. In 4 cases it was the propositus, in 5 the partner.

From the genetic point of view it is of some interest to note that of the three

monozygotic pairs in which one or both twin partners had spina bifida occulta, two were discordant in respect of this sign.

Discussion

In considerations of aetiology and pathogenesis of enuresis, the twin research method offers some fundamental advantages, in as much as it enables the investigator to keep some important relevant factors under control. The present study is based on a randomly selected series of twin children with nocturnal enuresis, using the twin partners as controls. The aim of the investigation was to ascertain whether there was any relationship between disorders of the lower urinary tract as revealed by micturition urethro-cystography and the occurrence of nocturnal enuresis. In order to avoid prejudicing influences in assessing the findings demonstrated by urethrocystography the examiner was not informed whether the child was affected with enuresis or not. Since in addition, possible biasing factors were especially taken into account in the calculations, it is considered that the analysis enables certain conclusions to be drawn which are presumably to some extent generally applicable to enuretic children.

The analysis has failed to reveal any significant associations between nocturnal enuresis, on the one hand and the width of the urethra or the effective capacity of the bladder on the other hand. Nor could any significant relationship be established between enuresis and minor functional or anatomical derangements of the lower urinary tract i.e. deficient opening of the bladder neck or the external sphincter or the presence of a non-obstructive transverse fold in the posterior urethra. Moreover severely pathological changes in the lower urinary tract were not found in any case. Thus the result does not provide any support to the assumption of some previous authors of such relationships to exist. Previous studies, however are not based on adequately controlled series. On the other hand, the frequency of minor deviations in the lower urinary tract is, in the present series higher though not significantly in the group of twins with persistent nocturnal enuresis than in the group of their partners. It is, therefore possible that a larger series would reveal a significant relationship between such deviations and nocturnal enuresis. The present study however indicates that such a relationship if it exists is not strong at least as far as *children* are concerned.

Summary

1 A study by means of micturition urethro-cystography of changes in the lower urinary tract and of the effective bladder capacity in children with nocturnal enuresis was performed on the basis of a randomly selected twin series. The material comprised 40 same-sexed twin pairs in which at least one of the partners suffered from persistent nocturnal enuresis, i.e. 49 affected twins and 31 unaffected partners who were used as controls.

2. Only minor deviations in the lower urinary tract were found. The investigation failed to show any significant relationship between such deviations and nocturnal enuresis.

TABLE 1 *Vitamin B_1, vitamin B_2 and vitamin C excretion in premature infants*

Case	Age* in days	Weight in grams	Vitamin B_1 excretion in % 1 day	2 day	3 day	Average	Vitamin B_2 excretion in γ 1 day	2 day	3 day	Average	Vitamin C excretion in mg 1 day	2 day	3 day	Average
1	3–5	2400	<1	<1	<1	<1	0.0	—	0.0	0.0	1.35	—	1.23	1.30
2	4–6	2900	<1	<1	<1	<1	0.0	11.0	18.0	9.7	2.60	2.63	1.37	4.20
3	5–7	1800	<1	<1	<1	<1	0.0	0.0	16.0	5.3	—	0.75	0.93	0.67
4	7–9	1800	<1	<1	<1	<1	17.0	17.0	20.0	18.0	3.23	1.30	1.25	1.94
5	9–11	1500	<1	<1	<1	<1	0.0	0.0	0.0	0.0	1.63	0.20	0.50	0.75
6	13–14	2200	<1	<1	<1	<1	5.0	0.0	0.0	1.7	4.90	1.45	1.53	2.63
7	14–16	1600	<1	<1	<1	<1	0.0	0.0	0.0	0.0	—	—	0.77	0.77
8	21–23	2100	<1	<1	—	<1	22.0	23.0	—	22.5	1.90	—	—	1.90
9	29–31	2100	<1	<1	<1	<1	1_.0	12.0	1_.0	1_.0	4.93	_.5	1.61	_.43
10	32–34	1900	<1	<1	<1	<1	25.0	25.0	11.0	20.3	0.00	0.10	1.73	0.6_
11	32–34	2000	<1	<1	<1	<1	22.0	15.0	25.0	20.7	0.73	1.90	—	1.33
12	36–38	100	<1	<1	<1	<1	52.0	52.0	52.0	52.0	0.03	0.60	0.23	0.30
13	40–4_	2200	7.0	4.0	4.0	5.0	_5.0	30.0	25.0	26.7	0.45	0.85	1.60	0.97
14	41–43	1800	<1	<1	<1	<1	60.0	5_.0	60.0	60.0	0.55	0.00	1.03	0.53
15	62–64	2100	<1	<1	<1	<1	5.0	0.0	15.0	6.7	—	0.67	2.03	1.36
						<1				1_.4 ±4.7				1.45 ±0.73

* The age of the premature infant during the investigation. (Example: In case 10 the premature infant was 32 days old on day 1, 33 days old on day 2 and 34 days old on day 3.)

The general condition of the mothers at the time of delivery was good or moderately good. A corresponding comparison with the premature infants was not possible as they were usually born elsewhere and records of the mother's condition therefore often limited.

Discussion

Vitamins B and C play a significant role in the development of the fetus. In experimental work with animals diets low in vitamin B have been observed to cause multiple deformities in the fetus (15, 16, 30). In premature and newborn full term infants, vitamins B and C are also important in the metabolic processes of the organism (10, 19).

The vitamin depots of the newborn are definitely dependant on the diet and nutritional condition of the mothers during pregnancy (6). The placenta conveys vitamins B and C from the mother's blood to the fetus but evidently their transportation in the opposite direction is prevented (1_, 13, 2_). A resulting vitamin deficiency is not very common in the newborn. Among deficiency conditions in this period infantile beriberi is the most frequent and numerous reports on its occurrence have been published (31, 32, 34, 35). In Finland vitamin B deficiencies are rare owing to the present-day diet. In the United States lack of vitamin B_1 (_7) has been described in mothers under _0 years of age due to their somewhat one-sided diet (30). The vitamin B equilibrium may also be lightly disturbed during illness. The nutritional state of the mothers in

TABLE . Vitamin B_1, vitamin B_2 and vitamin C excretion in newborn infants.

Case	Parity no.	Hgb g %	Weight in kg	Length in cm	Complicating diseases	Birth weight in g	Vitamin B_1 excretion in γ %: 1. day*	2. day	3. day	Average	Vitamin B_2 excretion in γ %: 1. day*	2. day	3. day	Average	Vitamin C excretion in mg %: 1. day*	2. day	3. day	Average
1	I	11.8	61.5	161		3060	5.0	4.0	5.0	4.7	0.0	5.0	0.0	1.7	1.90	1.00	0.30	1.37
2	II	12.7	61.0	167		3130	28.0	<1.0	5.0	11.0	11.0	5.0	5.0	7.0	3.20	0.60	1.20	1.70
3	I	16.4	66.1	163		3220	<1.0	6.0	3.0	3.0	35.0	42.0	10.0	25.7	1.35	—	1.70	1.52
4	I	13.3	63.0	160		3250	16.0	24.0	2.0	14.0	12.0	6.0	0.0	6.0	4.00	10.15	1.30	5.15
5	III	13.7	71.0	160	Lues seropos. vs. med.	3430	8.0	4.0	—	6.0	40.0	12.0	—	26.0	2.85	1.00	—	1.92
6	I	13.7	67.5	158		3550	2.0	6.0	<1.0	2.7	33.0	0.0	0.0	11.0	2.00	2.00	1.15	1.72
7	II	11.8	76.5	167	Anaemia gravid.	3570	<1.0	14.0	20.0	12.0	18.0	25.0	5.0	16.0	2.45	0.00	12.35	7.93
8	I	10.5	72.5	160	Pyelonephritis gravid.	3600	2.0	2.0	1.0	1.3	62.0	10.0	0.0	24.0	10.80	2.15	4.20	5.72
9	III	11.5	88.0	165		3650	4.0	16.0	24.0	14.7	44.0	12.0	10.0	22.0	14.90	2.20	8.00	8.37
10	III	12.3	67.0	162		3650	<1.0	<1.0	1.0	<1.0	0.0	0.0	0.0	0.0	1.15	1.55	2.75	1.82
11	IV	15.0	75.0	168	Influenza	3670	—	2.0	<1.0	1.5	—	0.0	0.0	0.0	—	2.90	2.15	2.52
12	I	11.8	85.0	155	Prae-eclampsia gravid.	3800	6.5	2.0	1.0	2.5	68.0	20.0	0.0	29.3	0.80	1.65	3.50	1.92
13	I	14.5	74.0	163	Hypertensio gravid.	3900	6.5	12.0	2.0	[illegible]	52.0	20.0	0.0	24.0	—	3.50	3.05	3.28
14	V	12.7	81.5	162	Cholecystectomia	3900	6.0	2.0	—	4.0	28.0	4.0	—	16.0	1.70	—	—	1.70
15	VII	12.7	101.0	165	Hypertensio gravid.	3920	28.0	28.0	30.0	28.0	60.0	5.0	5.0	23.0	3.55	2.85	3.60	3.33
16	III	12.9	74.0	165	Hypertensio arterialis.	3950	4.0	11.0	—	7.5	35.0	40.0	—	37.5	3.30	4.05	—	3.68
17	I	8.7	66.9	164	Anaemia grav. d. Thrombophlebitis superf. et prof. crur. l. sl.	4000	<1.0	3.0	—	2.0	35.0	16.0	—	25.5	3.60	5.65	—	4.63
18	VIII	11.8	71.8	156		4020	20.0	12.0	3.0	15.0	75.0	15.0	0.0	[illegible]	3.05	3.45	[illegible]	2.23
19	VI	13.3	65.0	161	Influenza	4040	5.0	10.0	6.0	7.0	5.0	30.0	15.0	16.7	4.00	7.30	8.10	6.47
20	I	10.5	74.7	168	Pyelonephritis gravid. Hypertensio gravid.	4150	15.0	50.0	90.0	51.7	90.0	52.0	11.0	51.0	5.05	4.90	8.65	6.73
21	IV	11.5	67.0	161		4150	3.0	5.0	—	4.0	50.0	25.0	—	37.5	2.75	5.00	—	3.88
22	I	12.3	67.6	162		4240	9.0	<1.0	<1.0	3.7	5.0	5.0	5.0	5.0	7.65	1.60	1.10	3.45
23	I	12.5	77.4	167	Prae-eclampsia gravid.	4250	16.0	16.0	16.0	16.0	25.0	18.0	16.0	19.7	10.70	5.40	9.55	8.55
24	III	11.5	72.5	166	Influenza	4570	6.5	—	16.0	11.3	112.5	—	12.0	62.3	10.40	—	21.40	15.90
							8.9	10.4	11.1	9.6 ±2.2	20.3	16.3	4.9	21.9 ±3.1	4.63	4.09	5.03	4.54 ±0.68

* 1, 2 and 3 denote the first three days of life.

TABLE I *Vitamin B_1 vitamin B_2 and vitamin C excretion in premature infants.*

Case	Age* in days	Weight in grams	Vitamin B_1 excretion in % 1 day	2. day	3. day	Average	Vitamin B_2 excretion in γ 1 day	2. day	3. day	Average	Vitamin C excretion in mg 1 day	2. day	3. day	Average
1	3– 5	2400	< 1	< 1	< 1	< 1	0.0	—	0.0	0.0	1.35	—	1.[illegible]3	1.30
2	4– 6	2900	< 1	< 1	< 1	< 1	0.0	11.0	18.0	9.7	2.60	8.63	1.25	4.29
3	6– 7	1800	< 1	< 1	< 1	< 1	0.0	0.0	16.0	5.3	—	0.75	0.98	0.87
4	7– 9	1600	< 1	< 1	< 1	< 1	17.0	17.0	20.0	18.0	3.[illegible]3	1.30	1.28	1.94
5	9–11	1800	< 1	< 1	< 1	< 1	0.0	0.0	0.0	0.0	1.05	0.20	0.89	0.71
6	12–14	2200	< 1	< 1	< 1	< 1	5.0	0.0	0.0	1.7	4.90	1.45	1.63	2.63
7	14–16	1800	< 1	< 1	< 1	< 1	0.0	0.0	0.0	0.0	—	—	0.77	0.77
8	21–23	2100	< 1	< 1	—	< 1	22.0	25.0	—	23.5	1.90	—	—	1.90
9	29–31	2100	< 1	< 1	< 1	< 1	12.0	12.0	12.0	1[illegible]0	4.93	2.25	1.03	2.42
10	3[illegible]–34	1900	< 1	< 1	< 1	< 1	[illegible]5.0	25.0	11.0	[illegible]0.3	0.00	0.10	1.75	0.62
11	3[illegible]–34	2000	< 1	< 1	< 1	< 1	22.0	18.0	23.0	[illegible]0.7	0.75	1.90	—	1.33
12	36–38	2100	< 1	< 1	< 1	< 1	5[illegible]0	52.0	52.0	52.0	0.05	0.60	0.23	0.29
13	40–4[illegible]	2200	7.0	4.0	4.0	5.0	25.0	30.0	28.0	26.7	0.45	0.88	1.60	0.97
14	41–43	1800	< 1	< 1	< 1	< 1	60.0	52.0	68.0	60.0	0.55	0.00	1.03	0.53
13	82–84	2100	< 1	< 1	< 1	< 1	5.0	0.0	15.0	6.7	—	0.67	2.03	1.35
						< 1				17.4 ± 4.7				1.43 ± 0.79

* The age of the premature infant during the investigation. (Example: In case 10 the premature infant was 32 da[ys] old on day 1, 33 day[s] old on day [2] and 34 days old on day 3.)

The general condition of the mothers at the time of delivery was good or moderat[el]y good. A corresponding comparison [w]ith the premature infants was not pos-sible as they were usually born elsewhere, and records of the mother's condition therefore often limited.

Discussion

Vitamins B and C play a significant role in the development of the fetus. In experimental work with animals, diets low in vitamin B have been observed to cause multiple d formities in the fetus (15 16 30). In premature and newborn full term infants, vitamins B and C are also important in the metabolic processes [o]f the [o]rgani[s]m (10 10).

The [v]itamin [d]epot[s] [o]f th[e] newborn are definitely dependant on the diet and nutritional condition of the mothers dur-ing pregnancy (6). The placenta conveys vitamins B and C from the mother's blood to the fetus, but evidently their trans-portation in the opposite direction is pre-vented (1* 13 *7). A resulting vitamin deficiency is not very common in the new-born. Among deficiency conditions in this period infantile beriberi is the most fre-quent and numerous reports on its oc-currence have been publi[s]hed (31 32, 34 35). In Finland, vitamin B deficiencies are rare owing to the present-day diet. In the United States lack of vitamin B (*7) has been described in mothers under *0 years [o]f age due to their somewhat one-sided diet (30). The vitamin B equilibrium may also be highly disturbed during illness. The nutritional state of the mothers in-

delivered by a highly anemic mother (Case 17) was found to be low The vitamin B_1 excretion in many other children, however was still lower and no distinct relationship between these occurrences can be made. Diseases complicating pregnancy i.e pre-eclampsia, hypertensio gravidarum and influenza, did not influence the infant s vitamin excretion either

Comparing the results obtained in this investigation, it was observed that practically no vitamin B_1 excretion occurred in premature infants, regardless of age and weight. In any event, in none of the cases were vitamin B_1 deficiency symptoms clinically noted. These observations agree well with previously published studies, and the clinical reports that B_1 avitaminosis is the most common of the vitamin deficiencies in the newborn. It may be reasonable to bear in mind that infantile beriberi could appear even in this country

The excretion values for vitamin B_2 in premature infants correspond completely with those given in previous reports. The danger that deficiency states would develop does not seem to be great although considerable differences are observed between the various cases in our material The values for vitamin C excretion are low The observation that vitamin C excretion in premature infants over three weeks of age despite administration of vitamin C, is less than in younger infants strongly supports the necessity of vitamin C administration.

Values for the excretion of Vitamin B_1 in the urine of newborn full term infants remain high and relatively unchanged in the three first days of life. The average excretion values correspond to those given by Brock (*) The abundant excretion of vitamin B_2 agrees well with Neuweiler s (18) observations. The excretion values for vitamin C remain, on an verage equally high on the first three days of life.

The weight of the newborn at birth was not noted to have any distinct influence on the vitamin content in the urine The same observation was made in premature infants.

Summary

The daily excretion of vitamins B_1 B_2 and C was studied in 24 newborn full-term infants during the three first days of life and in 15 premature infants on three successive days of treatment. No correlation was observed between the mothers state of health during pregnancy the number of previous births and the presence of certain diseases with the daily excretion of vitamin B_1 B_2 and C in the urine of newborn full-term infants.

References

1 ANDERSON N A.: Care of the premature infant. Mitchell-Nelson Textbook of Pediatrics. Fifth Edition. Saunders, Philadelphia 1950.

2. BROCK, J Biologische Daten fur den Kinderarzt Springer Berlin II Band; 390 1954

3 DEAN R. F A and HOLMES W I M Excretion nd intake of B vitamins in newborn infants *Arch Dis Child* 25 792, 1950.

4 DUNHAM, E. C Premature Infants. Hoeber Harper New York. 1955.

5. Food and Nutrition Board, National Research Council: Recommended Dietary Allowances. Revised 1958.

6 HAMIL, B., MUNKS, B., MOYER, E., KAUCHER M and WILLIAMS, H.: Vitamin C in the blood and urine of the newborn and in he cord and maternal blood. *Amer J Dis Child* 74 417 1947

7 HAMIL, B., CORYELL, M., RODERUCK, C

Kaucher, M., Moyer, E., Harris, M. and Williams, H. Thiamine riboflavin, nicotinic acid, pant thenic acid and biotin in the urine of newborn infants. Amer J Dis Child 74 434 1947

8. Knott E. M Determination of vitamin B_1 requirement of infants by means of urinary excretion of thiamine. Proc Soc Exp Biol Med, 45 765, 1940

9 Lawrence, J Herrington B and Maynard L.: Comparative values of bovine and human milks in infant feeding. Amer J Dis Child, 70 193 1945

10 Levine, S. Z Gordon H H. and Marples, E. I. Defect in the metabolism of tyrosine and phenylalanine in premature infants. II Spontaneous occurrence and eradication by vitamin C. J Clin Invest, 20 209, 1941

11 Litchfield H R., Lichtenman J Knoll, I. and Kurland I Effect of yeast tract (Vitamin B complex) on growth and development of premature infants. Amer J Dis Child 57 546, 1939

12 Lund C. and Kimble, M.: Some determinations of maternal and plasma vitamin C levels. Amer J Obstet Gynec, 46 635, 1943.

13 Lust J E. Ha[illegible] D D and Miller, C. A.: The transport of riboflavin by human placenta. J Cli Invest 33 38, 1954

14. Melnick, D Vitamin B_1 (thiamine) requirement of man. J Nutr 24 139 1942.

15. Nelson M. M., Baird, C. D C., Wright H. V and E[illegible], H. M Multiple congenital abnormalities in the rat resulting from riboflavin deficiency induced by the antimetabolite galactoflavin. J Nutr 58 125, 1956

16 Nelson M M.: Production of congenital anomalies in mammals by maternal dietary deficiencies. Pediatrics 19 Suppl 4 764 1957

17 Nelson W E.: Textbook of Pediatrics. Sixth Edition. Saunders, Philadelphia–London 1954

18 Neuweiler, W Refer by Dean & Holman.

19. Nitowsky H M., Gordon C D and Gordon H H. Effect of hemopoetic and other agents on the hydroxyphenyluria of premature infants. Amer J Dis Child 85 462, 1953.

20 Roderuck, C Coryell, M Williams, H and Macy I. Free and total riboflavin content of colostrum and mature human milk. Amer J Dis Child 70 171 1945.

21 Roderuck, C Williams, H and Macy I Free and total thiamine contents of colostrum and mature human milk. Amer J Dis Child 70 162, 1945.

22. Sarett H P and Cheldelin V H.: Use of lactobacillus fermentum 36 for thiamine assay J Biol Chem 155 153, 1944.

23. Schaffert R. R. and Kingsley G. R.: Rapid, simple method for determination of reduced, dehydro- and total ascorbic acid in biological material. J Biol Chem 212 59, 1955

24 Selleg, J and King, C. G Vitamin C content of human milk and its variation with diet. J Nutr 11 599, 1936.

25. Slobody L., Benson R. and Mestern, J A comparison of the vitamin C in mothers and their newborn infants. J Pediat, 29 41 1946.

26. — A comparison of vitamin C in mothers and their premature newborn infants. J Pediat, 31 333, 1947

27 Slobody L., Willner, M. and Mestern, J Comparison of vitamin B levels in mothers and their newborn infants Amer J Dis Child, 77 736, 1949

28. Smith, C. A.: The Physiology of the Newborn Infant Third Edition. Blackwell Scientific Publications, Oxford. 311 319, 1959.

29 Snell, E. E. and Strong, F M.: Microbiological assay for riboflavin. Industr Engng Chem (Anal) 11 346, 1939.

30 Stearns, G N tritional state of the mother prior to conception JAMA, 168 1655 1958.

31 Stevenson S S.: Possible ariboflavinosis in a premature infant. Yale J Biol Med, 14 403, 1942

32. Toverud K U The xcretion of aneurin in pregnant and lactating women and in infants Z f. tam forsch, 19 251, 1930.

33. — Beret ung Om De Forste 6 Ars Arbeid Ved Oslo Kommunes Helsestasjon For Mor Og Barn På Sagene (1939–1944) Fabritius and Sons, Oslo, 1948.

34 Toverud K. U Stearns, G. and Macy I G Maternal utrition and child health. An interpret tive review Bull Nat Res Coun (Wash) 123, 1950

35. Van Gelder, D and Darby F Congenital and infantile beriberi J Pediat 25 226, 1944

36. Warkany J and Nelson R. C.: Appearance of skeletal abnormalities in the offspring of rats reared on deficient diet Science 92 383, 1940.

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Growth Changes in the Mongoloid Head[1]

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There has been a rather lively discussion [on] the form, size and growth of the head in mongoloid children (see f.i. Benda (4, 6)). As a contribution to the study of this question this paper which is concerned with growth changes involving the head in white Australian mongoloids, will be presented.

Material and Methods

Our findings are based on data obtained during a study of 6? male and 61 female mongoloids which included 9 male and 15 female adults (Tables 1, [2] and 3). These mongoloids are second generation Australians living in Melbourne.

The maximum head breadth, the maximum head length, and the horizontal head circumference have been measured according to the instructions of Martin & Saller (13). All measurements have been recorded to the nearest millimetre.

Findings and Discussion

1. *Maximum head breadth*

(Tables 1 and 2) Figure 1 records the means obtained during this investigation, in addition to those of Provis & Ellis (10) and Westropp & Barber (23) for normal children. The data of these workers have been selected because the children they studied were well nourished and were racially similar to those included in the present study.

Male mongoloids. The average maximum head breadth lies within the normal range until the age of about one year after which it increases more slowly than in normal children until the age of five years. Between the ages of five and nine years this measurement increases more rapidly though somewhat irregularly than the average for the normal groups so that by the ninth year the average maximum head breadth is less than 1.0 S.D. below the normal average. After this age however there is no progressive increase as groups of older mongoloids are considered, whereas a continuing gradual increase is apparent in groups of normal children.

Female mongoloids. The average maximum head breadth is equal to that of normal females until the age of 0.? years but between this age and 4.5 years there is much less rapid increase in the mongoloid groups following which the mongoloids increase at a rate similar to that of normal children although somewhat irregularly. One mongoloid had a maximum head breadth markedly in excess of the normal average. These findings should be compared with those of Benda (4, 5) who

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TABLE 1 *Male mongoloids*

All measurements are in centimetres.

Chronological age years			Horizontal head circumference		Maximum head breadth		Maximum head length		Breadth/length index
Mean	S.D.	Number	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean
0.1	0.03	2	35.8	1.66	9.9	0.07	12.2	0.57	[illegible]
0.5	0.03	3	39.6	1.63	11.2	0.38	[illegible]	0.70	[illegible]
0.7	0.07	6	40.7	1.07	11.8	0.14	13.0	0.57	91.2
1.3	0.25	4	43.5	[illegible]	12.5	0.55	14.2	0.26	[illegible]
[illegible]	0.09	9	45.5	1.33	[illegible]	0.34	14.6	0.41	[illegible]
3.0	0.23	3	44.9	1.01	12.7	0.15	14.8	0.26	[illegible]
3.4	0.15	3	45.3	1.74	13.2	0.90	14.3	0.32	91.9
3.8	0.06	2	45.7	2.26	[illegible]	0.00	15.8	1.27	[illegible]
4.7	—	1	44.6	—	13.2	—	14.6	—	90.4
5.3	0.44	6	47.9	1.35	12.6	0.40	15.7	0.65	[illegible]
6.7	0.4	5	48.1	1.92	13.4	0.71	15.9	0.45	[illegible]
8.7	0.33	2	49.4	1.18	14.0	0.07	[illegible]	0.21	[illegible]
9.5	—	1	48.6	—	14.4	—	16.2	—	[illegible]
11.4	—	1	51.1	—	14.6	—	16.5	—	[illegible]
12.5	0.46		50.3	1.97	14.3	1.14	16.6	0.30	[illegible]
14.6	1.1	3	51.2	1.90	14.6	1.00	16.6	0.31	[illegible]
15.8	0.01	2	48.5	2.20	14.0	—	16.3	1.38	[illegible]
16.6	—	1	50.5	—	14.6	—	16.4	—	[illegible]
17.4	0.32		52.4	1.41	14.4	0.28	17.4	0.21	[illegible]

TABLE 2. *Female mongoloids.*

All measurements are in centimetres.

Chronological age years			Horizontal head circumference		Maximum head breadth		Maximum head length		Breadth/length index
Mean	S.D.	Number	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean
0.1	—	2	35.7	0.83	9.8	0.21	11.9	0.38	[illegible]
0.3	0.03	2	37.6	0.42	10.8	0.14	12.1	0.42	[illegible]
0.5	0.07	3	38.7	1.46	10.9	0.61	12.2	0.45	[illegible]
0.7	0.17	4	40.7	0.68	11.7	0.28	13.2	0.17	[illegible]
1.1	0.32	3	42.1	1.56	11.9	0.30	14.1	0.85	[illegible]
1.5	0.15	6	[illegible]	1.16	[illegible]	0.65	13.9	0.72	[illegible]
[illegible]	0.21	3	42.0	3.02	13.0	0.72	14.2	0.81	[illegible]
[illegible]	0.09		43.2	0.49	12.2	0.14	14.5	0.07	[illegible]
3.5	0.07	2	43.1	4.23	12.4	0.07	14.0	0.42	[illegible]
5.3	0.37	4	47.3	2.50	13.3	0.64	15.7	0.34	[illegible]
7.0	0.44	2	47.0	2.83	13.3	0.07	16.0	1.35	[illegible]
8.4	0.59	2	47.5	1.76	13.3	0.50	15.9	0.04	[illegible]
9.3	0.34	2	50.1	0.78	13.0	0.14	16.6	0.14	72.4
10.1	—	1	47.0	—	15.8	—	15.0	—	101.3
11.5	0.53	5	48.0	0.53	13.4	0.37	16.1	0.23	[illegible]
[illegible]	0.41	4	48.7	1.32	13.6	0.28	16.2	0.81	[illegible]
12.6	0.34	4	48.1	3.30	13.3	0.93	16.1	1.03	[illegible]
14.2	0.20	6	49.1	1.68	13.8	0.39	16.4	0.73	[illegible]
15.3	—	1	51.8	—	14.6	—	16.5	—	[illegible]
16.1	—	1	51.7	—	12.9	—	14.9	—	[illegible]
17.5	0.13	3	48.6	[illegible]	13.0	0.31	16.4	0.15	

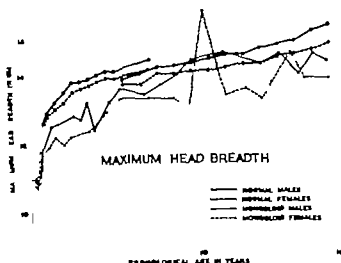


Fig. 1.

recorded a gradual rise in this measurement in his pooled data for male and female mongoloids.

Male and female mongoloids aged more than eighteen years (Table 3). The average values for the males are approximately equal to those for normal sixteen year-old boys while those of the females are pproximately equal to the average for normal girls aged thirteen years.

2. *Maximum head length*

(Tables 1 and 2 and Figure 2). During the first few months of life there is little difference between the average values in mongoloid and normal children of both sexes. This difference increases, however as progressively older groups are considered and reaches a maximum of -3.0 to -4.0 S D at about the age of five years. From this age until eight years the average

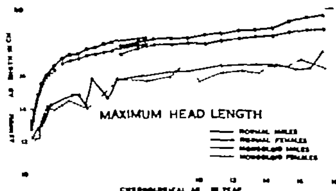


Fig. 2.

TABLE 3 *Head measurements on individual mongoloids aged more than 18 years*

All measurements are in centimetres.

Chronological age years	Horizontal head circumference	Maximum head length	Maximum head breadth	Breadth/length index
18.7 M	52.4	17.5	14.6	83.4
20.0 M	5[illegible].4	17.9	14.1	78.8
1.6 M	50.1	16.9	13.7	81.1
27.0 M	52.9	17.8	14.6	82.0
27.1 M	53.2	18.2	14.7	80.8
28.3 M	52.9	17.3	14.7	85.0
30.6 M	55.4	19.1	14.7	77.0
33.2 M	53.8	17.9	15.1	84.4
38.0 M	55.0	17.5	14.2	81.1
20.5 F	49.8	16.3	13.4	82.3
1.0 F	53.4	17.6	14.7	83.5
23.2 F	55.8	19.5	14.7	75.4
24.1 F	47.9	15.6	13.4	85.9
26.5 F	50.4	16.7	13.8	82.6
29.3 F	51.6	16.8	14.4	85.7
30.8 F	48.9	16.1	13.8	85.7
31.4 F	47.5	16.0	13.8	86.3
32.0 F	49.1	16.2	13.7	84.6
32.9 F	50.4	16.7	13.6	81.4
33.1 F	51.2	16.9	13.8	81.7
33.5 F	48.6	16.9	13.2	82.0
34.0 F	48.6	16.4	13.7	83.5
34.3 F	48.6	15.6	14.0	89.7
39.5 F	50.8	17.2	13.7	79.7

M = denotes male; F = denotes female.

values for both mongoloid and normal children increase at similar rates. These findings are in agreement with those previously reported.

Male and female mongoloids aged more than eighteen years. Few such mongoloids had a maximum head length in excess of the average for normal eight-year-old children (Table 3). However four males and five females had a maximum head length within the normal range (± 2 S.D.) for normal individuals of the same chronological age.

Some workers report an accelerated increase in head length in normal boys at about the age of 15 years (Boas (8); Wissler (24)) but no such change was observed by Boas & Wissler (9) or by Goldstein (11). Such a change was observed in the data for males at about the age of 17.4 years but because this is not a serial study it might indicate that male mongoloids with head lengths approaching normal values are more likely to survive.

In normal adults the maximum head length is positively correlated with body length (Martin & Saller 18) and the short stature of mongoloids might partly explain their low maximum head length.

3 *Breadth-length index*

(Tables 1 and 2, Figure 3). For all age groups except one the average breadth-length index is much higher than in normal

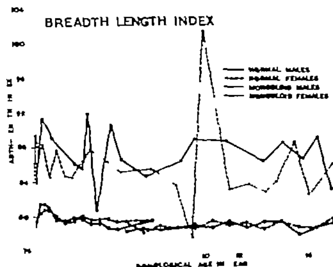


Fig. 2.

children even when due allowance is made for the small length of the mongoloid cranium. The index in both sexes rises in the first few months of life after which there are irregular but not progressive changes. These are similar to the changes reported for normal children (Westropp & Barber 23). As different age groups of mongoloid children are considered, mar ked fluctuations in the index are evident which do not bscure the high average inde in the mongoloid groups. Almost all these mongoloid children were brachy cephalic or hyperbrachycephali except for six males and ten females who were mesocephalic and one dolichocephalic male The index was equal to or in excess of 100 in four individuals.

Male nd female mongoloids aged more tha eighteen yea (Table 3). The adult males ha a mu h lower inde than the ounger males (t = 3.37 18 d.f.) but no such difference is pparent between the groups of females

It is difficult to determine why the maximum head length shows a greater variation from normal than the maximum head breadth. Howells (13) considers that the growth of the skull tends to be antero-posterior and that the growth of the brain tends to be transverse A view that the balance between these factors is responsible for the general form of the cranium, would lead us to conclude that in mongolism the rate of brain growth is more nearly normal than the rate of skull growth. Such a simplification of the problem is difficult to justify

The mongoloid cranium becomes abnormal in size and form during the first four years of life when the brain is growing rapidly and before the time of maximum muscular growth. This suggests that cere bral influences may be responsible for its bnormality Mongoloid children are more brachycephalic than normal children dur ing period when neither are able to support their heads and consequently in

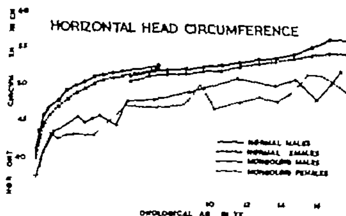


Fig. 4.

creased external occipital pressure cannot be wholly responsible for their brachycephaly

4 *Horizontal head circumference*

(Tables 1 2 and 3 and Figure 4) In both sexes the average circumferences follow normal levels until the age of one year From then until the fifth year they become progressively lower than those for normal children of corresponding age After this the rate of increase is approximately the same in the normal and mongoloid groups. Beyond 18 years the majority had a head circumference close to the average for normal children aged 7 to 8 years. These findings are in agreement with those reported by Benda (5). Contrary to the findings of Talbot (20) there were eight males and six females, some of them adults, with a horizontal head circumference within the normal range

Comment

It is clear that there are major variations from normal in the morphology of the mongoloid head. The etiology of these departures from normal must be considered in relation to the normal mechanism of head growth and the factors influencing them The observed variations may result from (a) retarded but otherwise normal growth, (b) an imbalance between normal growth processes, or (c) abnormal growth processes. This study has shown that at no stage can the head form be considered equivalent to that of a younger normal child.

There is experimental and clinical evidence that endocrine factors influence the morphology of the cranium The cranial changes in cretins and in animals following thyroidectomy (Losted et al. (14) among others) resemble those observed in mongoloids except that the head circumference of the latter is small proportional to body size

Benda (1956) claims that pituitary function is depressed in the mongoloid infant This view is supported by some experimental evidence (See 1 2 3, 10 19 *1) In addition hyperpituitarism in children is associated with increased cranial thickness and enlargement of superciliary arches and frontal sinuses (*7) which is

the reverse of what is common in mongolism (4 5 17 18) Such findings concerning thyroid and pituitary function are suggestive but they do not provide a complete understanding of mongolism. Other factors e.g. genetic ones cannot be excluded but a consideration of them is beyond the scope of this paper

Summary

1. The growth of the head has been investigated in 148 white Australian mongoloids.

2. The maximum head breadth was within the normal range (± S.D.) over approximately the first year In comparison with normal children, the increase for males was then less rapid until the age of five years, more rapid until the age of nine years, after which no continuing increase was observed. In the female group the changes were similar except for a slight increase throughout the period studied.

3. During first few months of life the maximum head length was almost normal It then increased much more slowly than in normal children until about the age of five years after which both groups showed approximately equal rates of increase

4 The breadth length index increased rapidly during the first few months of life to become much higher than in normal children, but variable. Most mongoloids are brachycephalic or hyperbrachycephalic, but examples of mesocephaly and dolichocephaly were observed.

5 The horizontal head circumference was normal until about the age of one year but between this and the fifth year it increased more slowly than in normal children after which the rate of increase was approximately the same

Acknowledgements

Our thanks are due to many who have helped during the course of this investigation, particularly the Mental Hygiene Authority of Victoria and their officers and the staff of the Anatomy Department.

References

1 Asling, C W Walker, D G., Simpson M E., Li, C H and Evans, H. M Deaths in rats submitted to hypophysectomy at an extremely early age and the survival effected by growth hormone *Anat Rec 114* 49, 1952.

2 Asling, C W Simpson M E Moon H. D Li, C H and Evans, H. M Growth hormone induced bone and joint changes in the adult rat The Hypophyseal Growth Hormone, Nature and Actions, p 144 McGraw Hill Book Company Inc New York, 1955

3 Asling, C W and Evans, H M.: Anterior pituitary regulation of skeletal development The Biochemistry and Physiology of Bone p 671 Academic Press Inc New York, 1956

4. Benda, C E.: Observations on the malformation of the head in mongoloid deficiency *J Pediat, 19* 800, 1941

5. — Mongolism and Cretinism, p 310. Grune and Stratton Inc New York, 1949.

6. — Mongolism. A comprehensive review *Arch Pediat 73* 391 1956.

7 Biedl, A.: Innere Sekretion, ihre physiologischen Grundlagen und ihre Bedeutung für die Pathologie p. 450 Urban & Schwarzenberg, Berlin, 1922.

8. Boas, F Changes in bodily form of descendants of immigrants. Reprinted from reports of the U.S. Immigration Commission N Y *1912*. (Quoted by Wunder 1930.)

9 Boas, F and Wissler, C.: Statistics of Growth. U.S Bureau of Education,

Report of the Commissioner of Education, Washington, 1905. (Quoted by Wissler 1930.)
10. Evans, H. M., Asling, C. W., Simpson, M. E. and Becks, H. The growth of hypophysectomized female rats following chronic treatment with pure pituitary growth hormone. IV Skeletal changes: Differences in response from that of intact rats. *Growth, 13,* 191 1949.
11. Goldstein, M. S. Development of the head in the same individuals. *Hum Biol 11* 197 1939.
12. Hjortsjö, C. H. and Lindegård, B.: Critical aspects on the use of indices in physical anthropology. *Kungl Fysiografiska sällskapets i Lund Förhandlingar 23* 1 1953.
13. Howells, W. W.: The cranial vault; factors of size and shape. *Amer J Phys Anthrop, 15* (n.s.) 19 1957
14. Lusted, L. B., Pickering, D. E., Fisher, D. and Smyth, F. S.: Growth and metabolism in normal and thyroid-ablated infant Rhesus monkeys (Macaca Mulatta). V Roentgenographic features of skeletal development in normal and thyroid-ablated infant Rhesus monkeys (Macaca Mulatta). *Amer J Dis Child 86* 426, 1953
15. Martin, R. and Saller, K.: Lehrbuch der Anthropologie 5th ed., Gustav Fischer Stuttgart, 1958.
16. Provis, H. S. and Ellis, R. W. B. An anthropometric study of Edinburgh schoolchildren. Part I. Methods, data and assessment of maturity *Arch Dis Child, 30* 328, 1955
17. Roche, A. F., Seward, F. S. and Sunderland, S.: Non-metrical observations on cranial radiographs in mongolism. To be published.
18. Roche, A. F. and Sunderland, S.: Post mortem observations on mongoloid crania. To be published.
19. Simpson, M. E., Van Dyke, D. C., Asling, C. W. and Evans, H. M.: Regeneration of the calvarium in young normal and growth hormone-treated hypophysectomized rats. *Anat Rec, 115* 615, 1953.
20. Talbot, F. B. Studies in growth. III. Growth of untreated mongolian idiots. *Amer J Dis Child, 28* 152, 1924
21. Walker, D. G., Asling, C. W., Simpson, M. E., Li, C. H. and Evans, H. M.: Structural alterations in rats hypophysectomized at six days of age and their correction with growth hormone. *Anat Rec 114* 19 1952.
22. Weinmann, J. P. and Sicher, H.: Bone and Bones. Fundamentals of Bone Biology p. 464 C. V. Mosby Co St. Louis, 1947
23. Westropp, C. K. and Barber, C. R.: Growth of the skull in young children. Part I Standards of head circumference *J Neurol Neurosurg Psychiat, 19* 52, 1956.
24. Wissler, C.: Growth of children in Hawaii based on observations of Louis R. Sullivan. *Memoirs of the Bernice P. Bishop Museum, 11* 257 1930.

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Bone Marrow Aplasia in Childhood Leukaemia

by ERNEST GLÜCK and JON LAMVIK

Hyperplasia of certain elements of the bone marrow and leukaemic infiltrations in the parenchymatous organs form the pathologico-anatomical basis of leukaemia and are the usual findings on post-mortem investigation. Occasional cases in which these findings are absent at post mortem have been reported previously Kirshbaum & Preuss (5) found that 14 out of 123 patients dying of leukaemia had a 'fatty marrow' at post-mortem. Since the antimetabolite drugs have been used in the treatment of leukaemia, several authors have published reports of bone marrow hypoplasia or aplasia (3 7 8 9 11) in some cases as a reversible phenomenon during treatment (8). It has been stated that the hypoplasia of the bone marrow can be a toxic effect of the preparation used (1 6 11). We have not been able to find any reports of terminal aplasia of the bone marrow in children with leukaemia

Between 1950 and 1959 inclusive 28 children with clinical diagnoses of leukaemia made on the basis of the usual clinical and haematological criteria were autopsied at the Gade Institute The bone marrow was

TABLE 1. *The haematological and post-mortem findings in six cases of leukaemia with hypoplastic or aplastic marrow*

			Initial haematological findings					Terminal haematological findings			Post mortem findings	
Case	Sex	Age years	Hb %	W.B.C./mm³ in 1000	Mononuclear cells %	Thrombocytes /mm³ in 1000	Bone marrow blast cell %	W.B.C./mm³ in 1000	Mononuclear cells %	Thrombocytes /mm³ in 1000	Bone marrow activity	Leukaemic infiltrations
1	M	13	39	142	93	0	97	9.7	33	298	Aplastic	Meninges
2	M	12	28	31	99	495	100	0.8	88	9	Hypoplastic	Absent
3	F	2	42	14	83	30	96[a]	1.6	100	0	Aplastic	Absent
4	M	2	58	12	84	3	70[a]	2.3	100	0	Hypoplastic	Absent
5	M	14	34	3	73	2	68	2.6	81	0	Aplastic	Absent
6	M	8	78	6	87	34	87	7.1	20	96	Hypoplastic[b]	Absent

[a] Low cellularity [b] With megaloblasts.

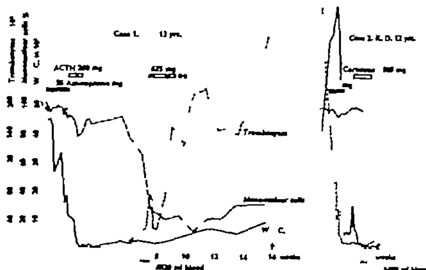


Fig. 1

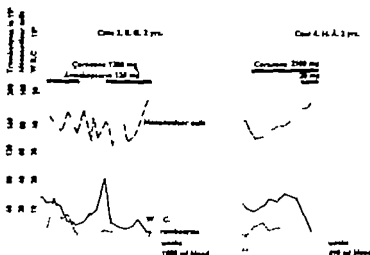


Fig. 2.

Figs. 1 and 2. Cases 1 .., 3 and 4 Treatment and variation in the haematological findings in the course of disease.

examined microscopically in 21 f these cases, of which six showed hypoplasia or aplasia of the bone marrow Some f the clinical, haematological and post-mortem findings in the latter gro p are shown in T ble 1 and Figs. 1 2. All the cases were classified as blast-cell leukaemia because the primit ve cell forms were so undiffer entiated Erythro- d thrombocytopenia were present in most of the patients at the time diagnosis was made Enlargement of the liver and spleen was present in all except Case 5.

All patients with a hypoplastic or aplastic marrow had been treated with folic acid antagonists, whereas 10 of 15 patients with a hyperplastic marrow had been so treated. In the patients with hypoplastic marrow the

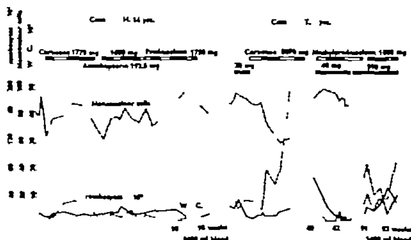


Fig. 3 Cases 5 and 6: Treatment and variation in the haematological findings in the course of disease.

length of the illness varied from 4 weeks to 22 months, reckoning from the onset of symptoms. In Cases 1 and 6 the course of the illness was interrupted by one and two clinical and haematological remissions, respectively. Terminal symptoms from the gastro-intestinal tract, such as stomatitis, vomiting, diarrhoea and persistent diffuse abdominal pain were present in all patients. Case 1 presented a progressive cachexia. A tendency to bleed was one of the commonest manifestations.

Post mortem investigation

Bone marrow examination from the lumbar vertebrae and the diaphysis of the

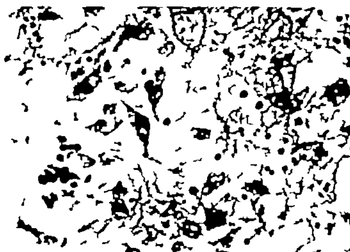


Fig. 4 Case 2. Hypoplastic vertebral marrow with scattered primitive reticulum cells. Left group of small mononuclear cells. (H E. ×480.)

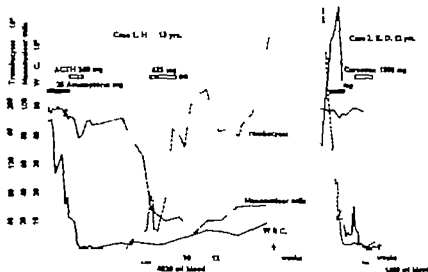


Fig. 1.

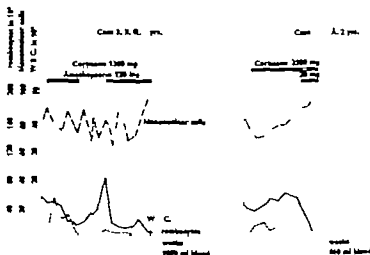


Fig 2.

Figs. 1 and 2. Cases 1 2 3 and 4 Treatment and variation in the haematological findings in the course of disease

examined macroscopically in 21 of these cases, f which six showed hypoplasia or aplasia of the bone marrow Some of the clinical, haematological and post-mortem findings in the latter group are hown in Table 1 and Figs. 1 2. All the cases were classified as blast-cell leukaemia because the primitive cell forms were so undiffer entiated. Erythro- and thrombocyt penia were present in most of the patients at the time diagnosis was made Enlargement of the liver and spleen was present in all except Case 5.

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a


b

Figs. 7 and 7b. Case 6: Macrophotograph of the testicle. *Left.* The biopsy 1 year before death, *right.* The testicular tissue at post mortem. (H. E. 350.)

(Cases and 4) there were more cells than in the previous group and were therefore termed hypoplastic (Fig 4) In Case 6 the cellularity of the vertebral marrow was apparently normal, but the picture wa dominated by megaloblasts, surrounded by some lymphocyte like cells (Fig 6) In this patient compact infiltrations of primitive mononuclear cells were found on testicular biopsy (Fig 7a) At post mortem these infiltrations were no longer present and the testes were fibrotic (Fig 7b) The same patient showed areas in both the ilium and the colon where the mucou membrane was replaced by a cellula granulation tissue which con tained remnants of metaplastic epithelium ith some synovtial areas.

Leukaemic infiltrations, most frequently in the liver spleen, kidney and lymph nodes, were found in all cases with a leukaemic bone marrow In five of the six cases with hypoplastic or aplastic marrow (Cases ., 3 4, 5 and 6) there were no leukaemic infiltrations in the organs exa mined. One child (Case 1) had an aplastic bone marrow and infiltrates of mononuclear cells in the meninges.

Discussion

It is evident from this analysis of 21 cases of clinically proved leukaemia that the process was still active when death occurred in 15 of the cases judging by the microscopic findings in the bone marrow In the remaining six cases there was a

distinct lack of leukaemic cell types in the marrow

Although there is always some concern that the microscopical sections are not representative the lack of leukaemic in filtrations in the organs together with the acellular bone marrow is a strong indication that our bone-marrow samples are representative and are indicative of the lack of activity in the leukaemic process.

The toxic effect of folic acid antagonists on the bone marrow is first a reduction of megakaryocytes followed by a reduction in the myeloid and erythropoietic elements (11) The myeloid elements are reduced more than the erythropoietic. The normal relationship between these, 4 1 may fall to less than 1 1 (12) Lymphoid tissue seems to be less affected. The commonest clinical manifestations of toxic effect are a tendency to haemorrhages and digestive tract symptoms, including stomatitis, glossitis vomiting, abdominal and diarrhoea. Ulcers may occur in the mucous membranes of the mouth and the intestines (11) Squamous metaplasia of the intestinal and bronchial epithelium has been seen in children treated with folic acid antagonists (13) In the bone marrow one may find an increase in the number of hypersegmented polymorphonuclear cells and giant types of meta myelocytes (1.) and megaloblasts may appear after a long period of treatment with aminopterin (2, 7 12) Leukaemic patients under treatment may have an increase in the differentiated granulocytes in the peripheral blood, in spite of the fact that the patient at the same time shows a reduction in the leucocyte count 8) The adrenocortical steroids and ACTH which are often used in conjunction with the folic acid antagonists may improve the regeneration of the normal marrow elements (6) As far as we are aware, hypoplasia of the bone marrow has not been reported following hormone treatment alone

The haematological changes and the post-mortem findings of the aplastic-hypoplastic group can be divided into several categories. In two of the patients (Cases 3 and 4) the bone marrow in spite of a moderate leucocytosis, showed low cellularity from the time the diagnosis was made, that is, before any treatment had been started. Both patients were 2 years of age One had had radium treatment for a haemangioma and the other was a mongol, both of which have a known relationship to the occurrence of leukaemia. Following the combined treatment with hormones and folic acid antagonists, a drop in the number of white blood cells and thrombocytes occurred but there was no haematological remission. It is reasonable to believe that the process in these patients was aplastic from the start and that the treatment may possibly have furthered this development. It is doubtful whether the folic acid antagonists can give a therapeutic advantage in patients in whom the bone marrow shows an aplastic tendency primarily

Two patients in this group (Cases and 5) had a very cellular bone marrow initially Folic acid antagonists did not, however give the expected haematological remission and the primitive cell remained proportionally very high during the entire illness In Case * there wa a considerable fall in the numbers of leucocytes and thrombocytes just after the folic acid

antagonists had been given. In this case there seemed to be a clear relationship between the treatment with folic acid antagonists and the haematological changes. Case 5 had marked thrombocytopenia and leucopenia during the whole course of the illness. The effect of the treatment on the development of the aplasia of the bone marrow was not directly evident No enlargement of the liver and spleen was ever noticed in this patient.

The last two patients in this group (Cases 1 and 6) had haematological remissions during treatment with folic acid antagonists. Case 1 showed the clinical signs of steady progression of the illness in spite of normalisation of the heamatological findings. The illness was characterized by severe pains in the extremities. A cachectic condition later developed with terminal stupor and coma. In spite of considerable enlargement of the liver and lymph nodes early in the illness there was no enlargement or leukaemic infiltrations at autopsy except for infiltrations in the meninges. Persistent leukaemic infiltration of the central nervous system in spite of haematological remission has been reported recently (4). This apparent resistance to treatment with folic acid antagonists is ttributed to lack of penetration by the drug, as it is thought that t s difficult for the drug to pass the blood-brain barrier The intrathecal administration of folic acid antagonist and steroid and X ray therapy ha been recommended in cases with central nervous system symptoms (4 10)

The dose of folic acid antagonists was small in Case 1 and wa stopped 3 month before death. However very small doses of folic cid antagonist have been reported to have a maximal and prolonged effect in cachectic patients who may have a deficiency of folic acid (11) On the other hand, large doses were used in Case 6 650 mg amethopterin spread over a period of 37 weeks, and the treatment continued right up to the death. In this patient intestinal changes characteristic of aminopterin intoxication were found at autopsy Further the bone marrow in both these patients showed a very intense megaloblastic reaction with giant forms, as can be found in folic acid deficiency

Summary

Six of *1 children with blast cell leukaemia were found t post mortem to have a hypoplastic or aplastic bone marrow Leukaemic infiltrations were found in the organs of all the cases with a hyperplastic marrow but were present in only one of the cases in the aplastic group and are limited to the meninges. The cause of aplasia of the bone marrow in leukaemia is discussed, particularly with regard to the possible influence of the folic acid antagonists. In two of the cases the bone marrow biopsies were indicative of an aplastic course from the start The treatment in these cases seems to hav reduced the activity of the bone marrow still further In one case the aplasia developed during prolonged treatment with folic acid antagonists, a causal relationship is, however uncertain. In the remaining three cases there was a haematological response lter treatment with folic cid antagonists. At utopsy changes that could have been due to lack of folic acid were found in the organs

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Two patients in this group (Cases and 5) had a very cellular bone marrow initially Folic acid antagonists did not, however give the expected haematological remission and the primitive cells remained proportionally very high during the entire illness. In Case 2 there was a considerable fall in the numbers of leucocytes and thrombocytes just after the folic acid

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Summary

Six of 21 children with blast cell leukaemia were found at post mortem to have a hypoplastic or aplastic bone marrow. Leukaemic infiltrations were found in the organs of all the cases with a hyperplastic marrow but were present in only one of the cases in the aplastic group and are limited to the meninges. The cause of aplasia of the bone marrow in leukaemia is discussed particularly with regard to the possible influence of the folic acid antagonists. In two of the cases the bone marrow biopsies were indicative of an aplastic course from the start. The treatment in these cases seems to have reduced the activity of the bone marrow still further. In one case the aplasia developed during prolonged treatment with folic acid antagonists; a causal relationship is, however, uncertain. In the remaining three cases there was a haematological response after treatment with folic acid antagonists. At autopsy changes that could have been due to lack of folic acid were found in the organs.

References

1 DAMESHEK, W and GUNZ, F.: Leukemia. Grune & Stratton. New York and London, 1959

2. HAMILTON L., PHILIPS, F S., STERNBERG S. S CLARKE, D A. and HITCHINGS, G H.: Hematological effect of certain 2,4-diaminopyrimidines, antagonists of folic acid metabolism. *Blood*, *9* 1062, 1954.

3. HAYHOE, F G and KOK A. Medullary aplasia in chronic myeloid leukaemia during busulphan therapy *Brit Med J* *1* 1468, 1957

4 HOVIND P Akutt myelogen leukemi (promyelocyttleukemi) med progredierende meningeal og cerebral affeksjon under hematologisk remisjon. *Nord Med* *62* 1821 1959

5 KIRSHBAUM J D. and PREUSS, F S.: Leukemia; clinical and pathologic study of 123 fatal cases in series of 14,400 necropsies. *Arch Int Med* *71* 777 1943.

6. PIERCE, M. I.: The acute leukemias of childhood, p. 497 *Pediat Clin N Amer* May 1957

7 SCHOENBACH, E. B. COLSKY J and GREENSPAN E. M. Observations on effect of folic acid antagonists, aminopterin and amethopterin, in patients with advanced neoplasms. *Cancer* *5* 1201 1952.

8. SMITH, C. H. and BELL, W R: Aminopterin in treatment of leukemia in children; serial aspirations of bone marrow as guide to management and appraisal of treatment. *Amer J Dis Child*, *79* 1031, 1950.

9. STICKNEY J M., HAGEDORN A. B., MILLS S. D and COOPER, T.: Changes in blood and bone marrow in acute leukemia induced by aminopterin. *J Lab Clin Med* *33*. 1481 1948.

10 SULLIVAN M. P.: Intracranial complications of leukemia in children. *Pediatrics*, *20* 757 1957

11 TAYLOR, S G HAAS, G M., CRUMRINE, J L. and SLAUGHTER, D. P Toxic reactions of 4-amino-pteroylglutamic acid (aminopterin) in patients with far-advanced neoplastic disease *Cancer* *3* 493, 1950.

12. THIERSCH, J B Bone-marrow changes in man after treatment with aminopterin, amethopterin, and aminoanfol, with special reference to megaloblastosis and tumor remission. *Cancer* *2*, 877 1949.

13 WESTON J T and GUIN G H.: Epithelial atypias with chemotherapy in 100 acute childhood leukemias. *Cancer* *8* 179, 1955.

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Venous Hypertension in the Newborn Infant Associated with Delayed Clamping of the Umbilical Cord[1]

by PAUL M. TAYLOR, THOMAS J EGAN EDNA L. BIRCHARD NANCY H BRIGHT and JEROME H. WOLFSON

In the course of studies of umbilical vein pressure in the newborn infant a temporary elevation of pressure was noted shortly after birth in several subjects. This report describes these observations, and further studies designed to test the hypothesis that this venous hypertension might be caused by rapid infusion of blood from the placenta associated with delayed clamping of the umbilical cord.

Patient Material

Umbilical vein pressure determinations ere obtained on a total of 62 infants during the first four days of life These infants have been divided into six groups:

Group A consists of 24 full-term and 7 premature infants on whom single determination of umbilical vein pressure was made Their ages ranged from 5 to 89 hours. None of the subjects in this group had clinical evidence of cardiac or respiratory difficulty Six of the premature infants were studied prior to the beginning of exchange transfusion for physiological jaundice The remaining infants in this group were chosen at random. The time at which the umbilical cord w clamped in these infants was not known

Infants in Group B through F were all delivered during the day and were considered to be mature. No other factors entered into the selection of these infants. Continuous bservations of umbilical venous pressure were made on these infants beginning shortly after the time of delivery and ending, in most cases, after the pressure had stabilized. A single repeat reading was made between the ages of 13 and 30 hours on infants in Groups B E, and F with the exception of one infant in Group B who had two repeat readings at 3 and 22 hours. The umbilical cord was clamped within a few seconds of birth on infants in Groups D and F and after a delay of several minutes on infants in Groups B C, and E.

Group B contains the six infants, all delivered *per vaginam*, on whom the initial studies f umbilical venous pressure at the time of delivery were carried out. These infants, with umbilical cords intact, were placed on table 30-45 cm below the level of the mothers perineum and the umbilical cord was manipulated and punctured almost immediately thereafter The time of clamping of the umbilical cord varied from 2–6 minutes after delivery in these infants. The umbilical cord was stripped i one case

Group C contains nine full term infants delivered *per vaginam* in whom a period of about 3 minutes elapsed between the time of delivery and clamping of the umbilical cord. The infants were placed upon a table 30-45 cm below the level of the mothers perineum,

[1] Supported by he Twenty Five Club Women Auxiliary of he Elizabeth Steel Magee Hospital

and were not handled except for gentle aspiration of secretions from the nose and throat; the umbilical cord was not manipulated during this period and care was taken to prevent it's being subjected to undue tension.

Group D consists of six full term infants delivered by the vaginal route whose umbilical cords were clamped within a few seconds of delivery during which time they were held above the mother's abdomen.

Infants in Groups E and F were delivered by low cervical cesarean section prior to onset of labor.

Group E contains five full term infants who were placed as low as possible on the operative field immediately after delivery. The placenta was then delivered and the infant and the placenta were removed together to a table upon which the infant was placed. The placenta was then held as far above the infant as feasible without traction being placed on the cord, with the fetal surface of the placenta facing the infant. This position was held for three minutes, or until the cord vein appeared collapsed. In four studies the cord was stripped throughout it entire length in the direction of the baby.

Group F consists of four full term infants and one premature infant whose umbilical cords were clamped at the time of delivery.

Estimation of the volume of blood remaining in the placenta following either early or delayed clamping was made on a separate group of 28 infants, all of whom were full term and delivered by the vaginal route.

Methods

Umbilical vein pressure was measured in mm of normal saline; values were read directly from a simple manometer. The saline contained approximately two units of Heparin® per ml. The infants were studied in the supine position. The mid-axillary line was chosen as point of reference for the manometer as it approximates the level of the right auricle. In the Group B infants the umbilical vein was punctured with a large needle, the needle withdrawn, and the umbilical vein catheterized through the rent in the vessel. Attempts to obtain pressure readings prior to cessation of the blood flow in the umbilical vessels were successful in but one of the six infants in this group. In infants in Group A and Groups C through F the umbilical vein was incised within cm of the abdomen prior to catheterization and polyethylene or polyvinyl catheter filled with saline was inserted into the umbilical vein until marked and rapid excursions of pressure occurred immediately with changes in the infant's activity. Withdrawing the catheter 2 or 3 cm from the point at which maximal fluctuations were noted did not modify the venous pressure reading. Measurements obtained at autopsy indicate that the junction of the umbilical vein and ductus venosus is found at a distance of 8–9 cm from the umbilicus in full-term infants. The majority of the venous pressure readings were obtained at depths of insertion (measured from the junction of the umbilicus and the abdominal wall) of 7 cm in the full-term infants, and of 6 cm in the premature infants. Occasional readings taken with the catheter inserted deeper than 8 cm were deleted. Hence it is believed that the values reported were obtained with the tip of the catheter in the recess of the umbilical vein.

The influence of crying and general muscular activity on the infant's umbilical vein pressure introduced the greatest possible source of error in these data. Readings were accepted only when the pressure was constant for at least several seconds, when the fluctuations normally seen with respirations were noted, and when the observers judged the infants to be maximally relaxed. Such an evaluation is necessarily somewhat subjective.

All infants were given intramuscular penicillin and dihydrostreptomycin for the three days following the umbilical vein catheterization.

Results

The mean umbilical vein pressure of 31 infants from 5 to 80 hours of age

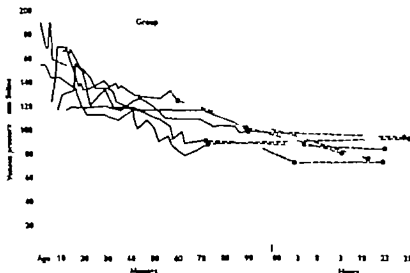


Fig. 1 Umbilical vein pressure patterns f infants with delayed clamping of the umbilical cord (Group B).

The umbilical cord was manipulated and punctured immediately after birth in these infants. Solid lines connect values obtained on initial insertion of the catheter Dotted lines connect the last of these alues and the single repeat reading or readings.

(Group A) was 9._2 mm of normal saline ± 1 .8 mm (S D). Age and birth weight did not influence the level of umbilical vein pressure. The highest pressure was 11. mm

Observations on the initial series of infants studied from birth (Group B) are shown in Figure 1 Ea h of these six infants had umbilical vein pressures above the range defined by the Group A infants at an older age five of the six had pressures in excess of 150 mm at some time during the first twenty minutes of life The highest pressure was recorded in the only subject in whom the umbilical vein was successfully catheterized with the cord intact and pulsating vigorously This in fant s umbilical ein pressure w 190 mm t two minutes f ge dropped to 170 mm ith the onset f respirations one minut later and then rose transiently to 190 mm following vigorous stripping of the umbilical cord. Clamping of the cord, immediately after the final stripping was followed by a drop in pressure to 160 mm within one minute

Continuous observation of umbilical vein pressure was made on four of these infants until their pressure had stabilized within the Group A range. Stabilization took place within 30–90 minutes of age Continuous recordings were abandoned while venous pressures were still elevated in two infants t 8 and 60 minutes of age Repeat determinations on these subjects at 3 nd 10 hours respectively were within the Group A range Three of the Group B infants breathed spont neously t birth, the remaining three from 3–6 minutes fter delivery The time f onset.

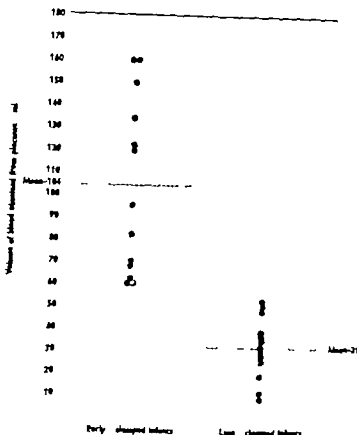


Fig. 2. Volume of blood obtained from the placenta in 14 early and 14 late-clamped infants.

of respiration did not seem to bear any clear relationship to the infant's venous pressure pattern.

An estimation was then made of the volume of blood transferred from placenta to full term infant during a three minute delay in clamping of the cord. It was assumed that the difference between the mean volumes of blood obtained from the placentas of series of early and of late-clamped infants would approximate the mean volume of the placental transfusion. The mean volumes of blood obtained from the placentas of 14 infants with cords clamped at birth, and of 14 infants with cords clamped three minutes after birth, were 104 ml ± 37 8 ml (S.D) and 31 ml ± 14.1 ml respectively (Figure 2). The difference between these means, 73 ml, is significant ($P < 0.001$) These early and late-clamped infants are comparable as to route of delivery (all *per vaginam*) mean birth weight (early 3330 g; late 3190 g) and range of birth weight (early ??00 to 3912 g late 2495 to 3865 g)

It seemed likely therefore that the infants in Group B received relatively large infusions of placental blood yet it occurred to us that the vigorous manipulation and puncturing of the cord that started immediately after birth might modify blood flow through the placental

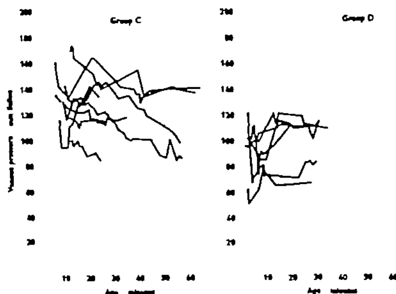


Fig. 3. Umbilical vein pressure patterns of early-clamped infants (Group D), and late-clamped infants (Group C) delivered vaginally Group C differs from Group B in that the umbilical cord of the Group C infants were not handled prior to clamping

vessels. Care was taken in the remaining studies to avoid touching or stretching the cord prior to its being clamped. Moreover an attempt was made from this time on to place the vaginally delivered infants at a uniform distance below the level of the mother perineum the studies of Duckman *et al.* (9) and Gunther (11) have emphasized that positioning the infant below the mother facilitates passage of blood from placenta to infant

Figure 3 allows comparison of the pressure patterns obtained on early-clamped (Group D) and intentionally late-clamped (Group C) infant delivered *per vaginam* The range of pressures recorded in the early-clamped infant i markedly lower than the range of values observed in the late-clamped group. The highest pressure recorded for the early-clamped infants was 120 mm seven of the nine late-clamped infants had venous pressures repeatedly in excess of 120 mm during at least the first twenty minutes of life

The study of infants delivered by repeat cesarean section prior to onset of labor was undertaken to exclude the possible influence of various factors associated with vaginal delivery on the infant s venous pressure. The infants in these groups breathed within a few seconds to 55 seconds after birth and were all in good condition at delivery Two infants in the early-clamped group (F) had minimal respiratory distress from birth, both were breathing normally by the age of 30 minutes. Figure 4 show that the early-clamped (Group F) infants venou pressures fall within the range of the Group A infant pressures. Four of the five infants in the late-clamped group (F) had pressures higher than the maximum venous pres-

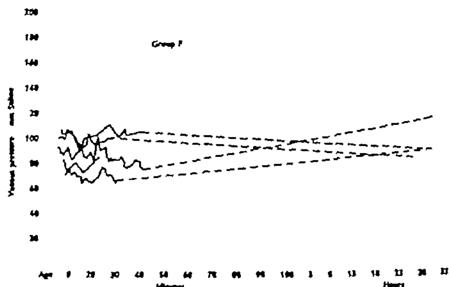


Fig. 4. Umbilical vein pressure patterns of early-clamped infants delivered by cesarean section.

sure of 110 mm recorded for Group F infants (Figure 5). Repeat single determinations were made on nine of these infants between 5 and 31 hours of age; all were within the normal range.

Discussion

The umbilical vein pressures of the Group A infants are within the range of the peripheral venous pressure reported for normal adults by Winsor & Burch (27) and for normal children reported by Burch (5) and Jacques (14), and are somewhat above the pressure reported by Lambert (16) for normal children. Bonham Carter, Bound & Smellie (3) found the mean umbilical vein pressure of normal infants during the first day of life to be 56 mm ± 12 mm (S.D.) using the sternal angle as point of reference for the manometer. We have found the mean difference between the mid-axillary line and the sternal angle to be 37 mm in full term infants and somewhat less in prematures. Addition of this difference to the mean value reported by Bonham Carter *et al.*, gives an adjusted mean of 63 mm. The difference between their adjusted mean value and our mean of 92.2 mm ± 12.8 mm is significant (t = 9.4) and unexplained. Our finding that venous pressure is not influenced by birth weight or by age is in accord with the observations of Bonham Carter *et al.*

The influence of early *versus* delayed clamping of the umbilical cord on the infant's venous pressure during the first minutes of life seems clear from our data. The eleven infants whose cords were clamped within a few seconds after delivery had umbilical vein pressure within or slightly above the range of pressure of the older Group A subjects. The maximum pressure seen in the early-clamped group was 140 mm; this value was recorded on two of the eleven infants. Elevation of venous pressure of the order of that noted in the initial series studied from birth

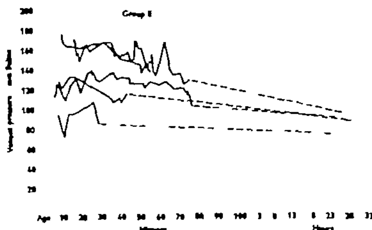


Fig. 5. Umbilical vein pressure patterns of late-clamped infants delivered by cesarean section.

(Group B) was recorded for most of the intentionally late-clamped infants; 11 of the 14 infants in Groups D and F had umbilical vein pressures higher than 1?0 mm. We have observed umbilical vein pressure in excess of 120 mm after the first hour or so of life only in infants with the clinical findings of congestive heart failure (25). Parkinson (20) has reported umbilical vein pressures of 80–98 mm H_2O during the first four minutes of life in a series of 14 normal infants whose cords were not stripped, and a pressure of 180 mm H_2O at the age of –3 minutes on one infant whose umbilical cord had been stripped.

The only consistent difference in management between the Group C and D subjects, and between the Group E and F subjects was the time following delivery at which the umbilical cord was clamped. Delay in clamping of the cord, under the conditions previously outlined, effects transfer of an average of 73 ml of blood from the placenta to the fetal circulation. Haselhorst & Allmeling (1) who recorded weight gain for successive minutes following delivery in 120 infants with intact umbilical cords, found an average gain of 70 g at the end of three minutes.

The extent of the average placental transfusion is best understood when expressed in terms of percent of the infant's blood volume prior to transfusion. Assuming the initial blood volume to be about 10 % of the infant's body weight then an infant weighing 3500 g would receive a ?0 % increase in blood volume during the first three minutes of life. According to the data of Haselhorst & Allmeling (1?) increases of about 14 5 and 1 % of the infant's initial blood volume take place during the first second, and third minutes after delivery respectively. Cutting *et al* (6) using 1 % saline or 5 % dextrose solution and Landis and his co-workers (17) using Ringer's solution have most closely approximated this rate of infusion under experimental conditions. They injected animals with these solutions at a rate sufficient to increase their blood volume from 3–7 % per minute. These animal

showed an abrupt rise in venous pressure beginning a few minutes after the start of the infusion. The fastest rate of intravenous injection in human experiments was reported by Sharpey-Schafer & Wallace (23) who injected either normal saline or serum at a rate sufficient to increase blood volume 2–2.5 % per minute. They observed a rise in venous pressure within 2–3 minutes from the start of the infusion. In view of these studies, it is not surprising that most of the late-clamped infants showed an elevated venous pressure shortly after birth.

The experimental data of Henry Gauer & Sieker (13) on men indicate that change in central venous pressure is directly proportional to change of blood volume induced by bleeding or transfusion. Our experience with the late-clamped infants delivered by cesarean section suggests that a correlation may exist between the volume of blood received by the infant elevation of his venous pressure The ... al cord was not stripped in one infant in Group F and there was no visible evidence of decompression of cord and placental veins during the time the placenta was held above the infant, his venous pressure was within the normal range. Two irresolute strippings of the cord were done on another infant in this group, his venous pressure was only slightly elevated. Manual compression of the placenta and vigorous stripping of the cord were carried out on the three other infants in this group until the upper portion of the umbilical vein appeared collapsed, all three showed marked elevation of umbilical vein pressure

The studies of Gauer & Henry (10) Sharpey-Schafer & Wallace (23), and Warren et al (26) indicate that the return of elevated venous pressure to normal following rapid infusion under experimental conditions is accomplished, in part at least by reduction of plasma volume DeMarsh, Windle & Alt (8) and Mollison & Cutbush (18) have shown that hemoconcentration takes place within the first several hours of life in late-clamped infants. Rycheck, Tylka & Taylor (22) found that serial hematocrits taken on late-clamped infants during the first hours of life show in general, a reciprocal relationship to the venous pressure patterns presented in this report, although marked variation in rate of hematocrit adjustment was noted, while the hematocrit of early-clamped infants changes very little over this period of time Different rates of adjustment of plasma volume could partially account for the observed differences in rates of decline of venous pressure in late-clamped infants with venous hypertension.

A rapid transfusion of sufficient volume to elevate venous pressure might influence the clinical course of the newborn infant. Budin (4) and Reynolds (21) advocate delayed clamping of the umbilical cord on the thesis that the infused blood benefits the infant by filling his expanding pulmonary vascular bed. Bonham Carter (2) suggests that an increase in cardiac output secondary to the placental transfusion might be effective in initiating expansion of alveoli in the newborn infant He accepts the evidence advanced by Jäykkä (15) which is purported to show that erection of the pulmonary capillary plexus secondary to increased pulmonary blood flow is an important factor in initiating expansion of the lungs. This seems unlikely however as Dawes et al. (7) have

shown that the initial inspiration preceeds increase in pulmonary blood flow in fetal lambs. Avery Frank & Gribetz (1) have demonstrated in experiments on excised lungs that the effect of vascular congestion might facilitate expansion to a limited degree, but was not of sufficient magnitude to initiate it. Moreover it is not certain that increasing right ventricular output in the apneic infant will increase pulmonary blood flow all of the increase might traverse the ductus arteriosus. It is possible, as suggested by Bonham Carter (2) that an increase in back pressure from the left auricle on the pulmonary capillaries secondary to the placental transfusion might lower the inspiratory force needed for initial expansion of the lungs.

Temporary elevation of venous pressure secondary to the placental transfusion might be harmful to the newborn infant in some circumstances. Elevation of capillary pressure would increase the possibility of significant hemorrhage into organs such as the brain, the lungs and the adrenal glands which may be traumatized during delivery Since capillary fragility is greater in the premature than in the mature infant, according to the data of Oehme & Haberland (19) and Ylppo (28) increase in capillary pressure t birth might be especially harmful to the premature infant.

Warren *et al* (26) have shown that rapid intravenous infusions elevate pulmonary capillary pressure in proportion to the increase in general blood volume in the normal human adult. The relatively large volume of the placental transfusion might increase pulmonary capillary pressure to the point of producing edema As alveolar edema probably preceed the actual pulmonary hyaline membrane it seems reasonable to consider the possible connection between certain cases of the neonatal respiratory distress syndrome and the placental transfusion. This suggestion is based on the assumption that increasing right ventricular output by rapid blood transfusion will increase pulmonary blood flow and pulmonary capillary pressure in the newborn baby This may not be true as functional patency of the ductus arteriosus may permit unequal distribution of the increase in blood volume between the systemic and pulmonary circuits.

Speculation will not decide whether the placental transfusion causes significant benefit or harm to the newborn baby Observations from this clinic on the influence of early *versus* late clamping of the umbilical cord on the clinical course of the newborn infant will be published elsewhere (4)

Summary

The mean umbilical vein pressure of 31 normal infants 5 to 89 hours of age was 92 2 mm of normal saline ± 12.8 mm (S D) Venous pressure was not influenced by age or birth weight.

Continuous recordings of umbilical vein pressure were made on 11 infants whose umbilical cords were clamped at the time of delivery and on 20 infants whose cords were clamped several minutes after delivery The highest pressure recorded in the early-clamped group was 120 mm of the 11 infants had this pressure Seventeen of the 20 late-clamped infants had levels of venous pressure repeatedly in xcess of 120 mm during the first 30–60 minutes of lif with return to the normal range t varying rates.

Possible effects of a placental transfusion sufficiently large to raise venous pressure on the clinical status of the newborn infant have been discussed briefly

Acknowledgement

We greatly appreciate the encouragement and guidance offered by Dr Edmund R. McCluskey and Dr Robert Klein during the course of this study W are also indebted to the Obstetrical Staff of the Elizabeth Steel Magee Hospital, and to the Nursing Staffs of the Delivery Room, Operating Room, and Newborn Nurseries for their active cooperation in obtaining the data presented in this report.

Mrs. Lial Ross kindly translated most of the papers from the German literature

References

1 AVERY MARY ELLEN FRANK, N R. and GRIBETZ, L: The inflationary force produced by pulmonary vascular distention in excised lungs. The possible relation of this force to that needed to inflate the lungs t birth. *J Clin Invest, 38* 453, 1959

2. BONHAM CARTER, R. E.: The architectural function of pulmonary capillaries. *Lancet 273* 1292, 1957

3 BONHAM CARTER, R. E., BOUND J P and SMELLIE, JEAN M.: Mean venous pressure in the first hours of life *Lancet 271* 1320 1956

4 BUDIN P A quel moment doit on practiquer la ligature d cordon ombilical? *Progr Med (Par)*, 3 750, 1875, and 4 2, 1876

5 BURCH, G E.: A Primer of Venous Pressure Philadelphia. Lea and F biger p 107 1950

6. CUTTING, R A., LARSON, P S and LANDS, A M Cause of death resulting from massive infusions of isotonic solutions. An experimental study *Arch Surg 38* 599 1939

7 D WES, G S., MOTT J C WIDDICOMBE, J G and WY TT D G Changes in the lungs of the newborn lam. *J Physiol 121* 141 1953

8. DEMARSH Q B WINDLE, W F and ALT H L Blood volume of newborn infant in relation t earl and lat clamping of umbilical cord *Amer J Dis Child 63* 1123 194_

9 DUCKMAN S., MERK H LEWIN x W Y nd RED ELIZABETH The importance of gravity in dela ed ligation of the umbilical cord. *Amer J Obstet Gynec 66* 1 14, 1953.

10 G TER, O H and HEN J P Beitrag zur Homöostase des extraarteriellen Kreislaufs.Volumenregulation als unabhängiger physiologischer Parameter *Kl Wsch 34* 356, 1956

11 GUNTHER, M. The transfer of blood between baby and placenta in the minutes after birth. *Lancet, 272* 1277 1957

12. HASSELHORST, G and ALLMELING, A: Die Gewichtszunahme von Neugeborenen infolge postnataler Transfusion. *Z Geburtsh Gynäk 98* 103, 1930.

13 HENRY J P G UER, O H. and SIEKER, H. O The effect of moderate changes in blood volume on left and right atrial pressures. *Circulat Res, 4* 91 1956.

14. JACQUES, L. H.: Venous pressure in children. *J Iowa Med Soc, 33* 294, 194_

15 JÄYKKÄ, S.: Capillary erection and lung expansion. An experimental study *Acta Pediat 46*, Supplement 112, 1957

16. LAMBERT J P Venous pressure in children. *Amer J Dis Child, 53* 1035, 1936.

17 LANDIS, E. M., B OWN, E F CTELL, M and WYRE, C.: Central venous pressure in relation t cardiac competence blood volume and exercise *J Cli Invest, 25* 237 1946.

18. MOLLISON P L., and CUTBUSH, MARY Hemolytic disease of the newborn. Criteria of severity *Brit Med J 1* 123 1949

19 OEHME, J and HABERLAND, R.: Die Kapillarresistenz bei Kindern und älteren Erwachsenen. *Ärztl Wschr 1* 673, 1937

20 PARKINSON R. P A study of venous pressure in the newborn infant *J Pediat, 50* 174 1957

21 REYNOLDS, S R. M Circulatory adaptations t birth and their clinical implications. *Amer J Obstet Gynec 70* 148, 1953.

_ RYCHECK, R. R TYLKA, ELI OR, V and T YLOR, P M Unpublished observations.

23. SHARPEY-SCHAFER, E. P and W LACE, JOAN Circulatory overloading following rapid intravenous injections. *Brit Med J 2* 303, 194_

24. Taylor, P. M., Bright, Nancy H. and Birchard, Edw. L.: The effect of immediate versus delayed clamping of the umbilical cord on mortality and respiratory rates of the newborn infant. In preparation.

25. Taylor, P. M., Wolfson, J. H., Bright, Nancy H., Birchard, Edw. and Egan, T. J.: Umbilical vein pressure in congestive heart failure in the newborn infant. In preparation.

26. Warren, J. V., Brannon, E. S., Weens, H. S. and Stead, E. A., Jr.: Effect of increasing the blood volume and right atrial pressure on the circulation of normal subject by intravenous infusions. *Amer. J. Med.* 4: 193, 1948.

27. Winsor, T. and Burch, G. E.: Use of the phlebomanometer: Normal venous pressure values and a study of certain clinical aspects of venous hypertension in man. *Amer. Heart J.* 31: 387, 1946.

28. Ylppö, A.: Zum Entstehungsmechanismus der Blutungen bei Frühgeburten und Neugeborenen. *Z. Kinderheilk.* 38: 32, 1924.

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Sleep Patterns in Infancy

A Study of One Infant from Birth to Eight Months of Age

by ARTHUR H PARMELEE, Jr

Sleep and wakefulness are perhaps the most dramatic contrasts in daily human behavior that everyone experiences and observes. Sleep or its opposite wakeful ness, are global complex patterns of behavior Detailed studies of both states and the transition from one state into the other will give valuable information about the functions of the nervous system as well as other physiological functions of the body

The most comprehensive review of studies sleep is that of Kleitman (14) but this co ers the period up to 1939 Subsequently there ha e been many studies, particularly relat e to the neurophysiology of arousal. These are reviewed in the reports f the Macy Conferences on Consciousness (1) the report f the Quebec Conference on Consciousness ( ) and a recent book entitled "The Waking Brain by Magoun (16)

Studies of the evolution of patterns of behavior in the maturing organism give us a perspective for the study of the complex behavior patterns of the mature organism We are able to follow the integration of the less complex primitive behavior pattern of the immature organ ism into the more complex patterns of the mature organism For man this mean observing the evolution of behavior from infancy to adulthood. Certainly the study of the evolution of sleep and wakefulness patterns of the infant and child may be expected to contribute to our understanding of these processes.

The most recent comprehensive reviews of sleep in infants and children are those of Hellbrugge, Lange & Rutenfrans (10), and Debré & Doumic (5) Gesell & Amatruda (7) have studied sleep in the premature and full term infant. Buhler & Hetzer (4) Kleitman & Engelmann (15), and Moore & Ucko (17), among others, have studied sleep in infancy using samples ranging from 10 t 200 infants. There are, in addition several detailed reports of the evolution of sleep patterns in just one or a very few infants (7-9, 13, 18, 21)

In general all of these studies indicated that the newborn or very young infant has frequent short sleep periods, and as the infant matures the short sleep periods consolidate into fewer and longer sleep periods and the longest tend to occur more frequently at night. The wakeful periods also consolidate and tend to concentrate in the daytime Most parents and some doctors have the impression that infant several month old sleep much less than newborn or very young infants. Kleit

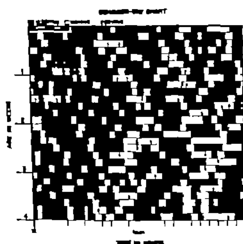


Fig. 1.

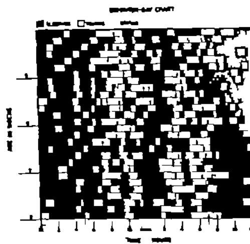


Fig. 2.

man & Engelmann (15) in their very careful study emphasize the fact that the change in total amount of sleep in 24 hours is not very great, the average total sleep being 14.8 hours at 3 weeks and 13 7 hours at *6 weeks. The major change is in the distribution of the sleep and wakefulness periods.

The material to be presented in this article concerns the behavior day records of one male child from birth to 35 weeks of age It seems justified to present this single case report since complete daily records of sleep and waking of a baby on a completely free schedule relative to eating, sleeping and waking, conscientiously kept by a mother for 35 weeks, are not readily obtained. Cases documented in such a manner can illustrat the general trend noted in the larger studies and at the same time emphasize individual varia tion.

Subject of the Observations

This male child was born after 38 weeks of gestation with a birth weight of 2385 g, height 47 cm, head circumference 3* cm. He cried immediately after birth and no physi cal or neurological abnormalities were noted in the neonatal period. His mother preg nancy had been normal. The labor lasted five hours and the delivery was that of a normal vertex presentation. Nitrous oxide was given to the mother at the time of delivery She received no medication during labor

He was fed on a completely self demand schedule. The self demand schedule extended to his sleeping in that he was never inten tionally awakened from sleep and every ffort was made t allow him t go t sleep when he desired. This started within the hour after his birth since he was in a rooming-in unit with his mother After the first eight days in this unit in the hospital, he slept in his own room which was large converted closet attached to the parent room. The large door to this room was left open and there was no intervening corridor His movements could be heard but he could not be seen from the parents room. H received primarily breast feedings with occasional supplemental cow milk feedings until 10 weeks f age From then on he received only cow milk. Purees were started in small amount t 15 weeks f age

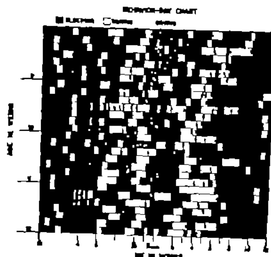


Fig 3

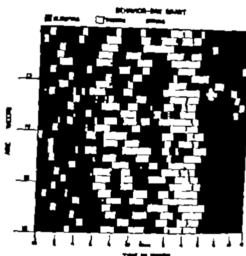


Fig 4

Physically and mentally he has progressed normally to the present. He is now twelve years of age.

Method

The mother of the subject recorded the infant daily activities on Behavior Day Charts as described by Gesell & Ilg (9) These charts have 24 vertical lines one-half h apart for the 24 hours f a day and l lines for recording each day s activities at the proper time interval. These horizontal lines are ⅜ inches apart, leaving ample room for recording There are 28 lines to each chart, thus one chart covers a four week period. In the wide margins of each chart is space for special notes relative to the growth f the child, special occur rences of the day or new developmental achievements, and so forth.

For this subject the mother made her daily recordings with colored pencils. Sleep was recorded as blue awake and quiet maroon crying red, breast feeding orange formula feeding yellow and water feeding violet. Since each interval between the vertical lines representing one hour of time measured one-half inch, the d ration of sleep and wakefulness periods co ld be recorded t the quarter hou with some accuracy

For the purposes of illustrating this article the Behavior Day Charts were reconstructed in black and white. In reproducing them in black and whit only the sleep (black) and wakefulness (white) are indicated. Crying, indicated by a vertical zigzag line, was only recorded during the first 16 weeks. The reproductions successfully illustrate the patterns of sleep.

The numerical data concerning the duration of total amount of sleep and wakefulness each day frequency of wakeful periods and longest sleep and wakeful periods, are derived from the original more detailed charts. The comments relati e t daily events and developmental progress are obtained from the mother s notes made daily on the original charts.

For the numerical values of durat n of sleep and wakefulness the first week of each chart was analyzed. For the first ek of life the total leep and wakefulness of the first day were not included because it was an incomplet day The longest sleep period f any day included the overlap nt the early morning of the next day since the intent on was to determine the abil ty of the infant t sustain leep and after the first weeks this period generally occurred at night and tended over midnight

TABLE 1 *An analysis of the infant's sleep during the first week recorded on each Behavior Day Chart including the first and last weeks of the total record*

(Times are given in hours.)

	(1) Total sleep per day — Av for week	(1) Total sleep per day — Range	(2) Av daily longest sleep per week	(3) Longest sleep period of the week
1st week	17.8	(16.5–20)	4.4	5.5
5th week	15.4	(14–16.5)	4.9	6.0
9th week	18.2	(15.5–20)	5.3	8.5
13th week	17.8	(16.5–19.5)	7.1	8.5
17th week	16.9	(16–18.5)	8.6	9.5
21st week	16.3	(15–17)	10.0	12.0
25th week	15.5	(13.5–17)	11.2	12.0
29th week	16.3	(15–17.5)	11.7	12.0
33rd week	14.4	(13–16)	10.7	12.5
35th week	14.3	(13–15.5)	7.9*	10.0

* Mother's notes state that baby was teething at this time and on Behavior Day Chart 9 it can be seen that there is a recurrence of wakening at night.

Discussion of the Behavior Day Charts

The numerical data presented in Tables 1 and 2 will be discussed first and then the Behavior Day Charts 1 through 9

In the 1st, 9th and 13th weeks[1] the total amount of sleep per day remained approximately the same The 5th week was an exception and this will be discussed later The total amount of sleep very gradually decreased in the succeeding week and in the 35th week it was only 3½ hours less than in the 1st week (Table 1 Column 1) The average duration of the longest sleep period per day increased rather rapidly with the most pronounced jumps between the 9th and 13th week and between the 17th and 21st weeks. The longest sleep period of each week followed the same general trend (Table 1 Columns and 3)

This boy therefore by the 21st week had developed the ability to sustain his sleep for periods of a little over twice the duration of those in the first week of life After the 21st there was not much change

The total amount of wakefulness was of course the inverse of the total amount of sleep. This gradually increased in duration proportional to the decrease in total amount of sleep (Table 1 Column 1)

The average longest wakeful period for each week studied was lower in length than was the average longest sleep period. By the 21st week the average longest wakeful period was [illegible] only 1.3 times that in the first week. Only in the 33rd week [illegible] jump to 4 hours which was [illegible] that in the first week (Table 2, Column [illegible]) Nevertheless there was a steady [illegible] the average

[1] The numbers of the weeks refer to the seven days preceding the corresponding numbers on the Behavior Day Chart Thus the first week refers to the seven days from birth to 1 and the 5th week to the seven days between the numbers [illegible] and 9 and so forth

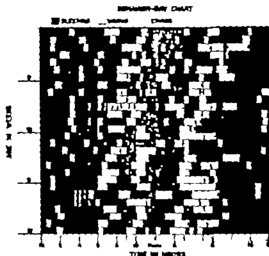


Fig 3.

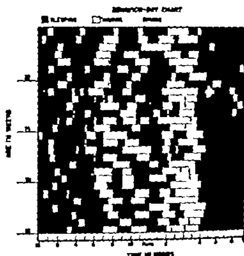


Fig. 4.

Physically and mentally he has progressed normally to the present. He is now twelve years of age

Method

The mother of the subject recorded the infant s daily activities on Behavior Day Charts as described by Gesell & Ilg (9) These chart have 24 vertical lines one-half part for the 4 hours of a day and zontal lines for recording each day s activities at the proper time interval. These horizontal lines are ⅜ inches apart leaving ample room for recording There are 28 lines to each chart thus one chart covers a four week period. In the wide margins of each chart is space for special notes relative t the growth f the hild, special occur rences f the day or new developmental achievements, and so forth.

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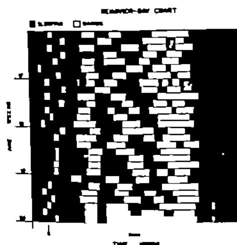


Fig. 5.

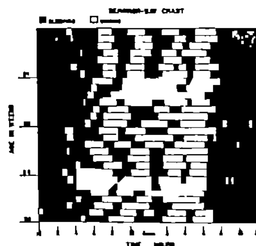


Fig. 6.

of time awake and also a greater fre quency of wakeful periods than in the first three weeks of lif He was also more fussy (Tables 1 and and Charts 1 and 2)

After the sixth week he was less restless and a diurnal sleep wakefulness pattern began to be apparent and became more obvious after the tenth week. From the 14th week on he slept consistently from 6 or 7 P.M. until midnight or beyond with rare relapses. At this time his afternoon schedule seemed to be quite regular also. His most disorganized period remained in the morning hours.

Crying was recorded on the Behavior Day Charts during the first 16 weeks and not after that. In the first 10 weeks crying was noted whenever there was prolonged wakeful period and especially in the afternoon and evening After 10 weeks the crying was less frequent and fter 1 eeks there were long wakef l periods without crying especially in the lat morning

This baby was breast fed for the first 10 wk rth some cow milk supplementa

After 10 weeks he received only cow milk feedings. Pureed foods and cereals were not started until after 16 weeks of age In the first 2 weeks of lif the frequency of feedings ranged from 6 to 8 per day with 7 the predominant number After the sixth week frequency of feedings was 5 to 6 per day with 5 per day most common after the 11th week.

From the 16th week on the major changes were in the "night waking between midnight and 4 A.M. as it is defined by Moore & Ucko (17) and in the day time sleep or naps occurring between 5 A.M. and 7 P.M.

The "night waking will be discussed first This boy had the least amount of night waking" from the 27th through the 33rd week. In this period he awakened between midnight and 5 A.M on 13 days of 4. or on an average of about two nights per week. During the *9th week (Chart 8) and for the first two days f the 30th week he did not awaken at ll from 6 P.M. until 5 A.M. This wa his most settled period and was the longest period of consecutive nights of prolonged

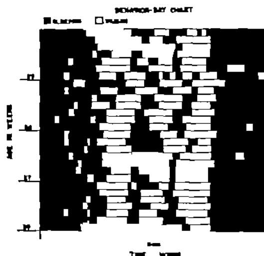


Fig 7

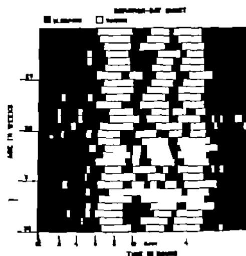


Fig. 8.

sleep His daytime sleeping periods were also very regular during the 29th week. From the 33rd through the 35th week (Chart 9) his "night waking" was more frequent again and there were irregularities in his day time sleep as well.

Moore & Ucko (17) considered a baby settled if he slept through from midnight to 5 A.M. Occasional lapses were accepted as long as they were less than once a week. In their analysis of 104 cases they found that approximately 70% had settled by three months of age and 80% by 4 months. Nearly 10% were not settled by the end of the first year. A large proportion of relapses were noted between the 6th and 10th months. Hellbrugge *et al.* (10) studied infant sleep by checking the cribs in an infant nursery every other hour. For a group of 12 infants 5–8 months of age, checked for a period of 80 days, they found only 5% awake at 9 P.M. after a scheduled feeding. However at 11 P.M., 1 A.M. and 3 A.M. 15% to 20% of the babies were awake and by 5 A.M. 35% had awakened.

The midday sleep or "naps" of this boy were irregular between 16 and 20 weeks (Chart 5). In general there were two or three "naps" of irregular length during the day between 5 A.M. and 7 P.M. By the end of the 20th week and during the 21st week (Chart 6) the baby developed a very regular three nap schedule. This became irregular again from the 22nd through the 25th week when a new schedule of two regular daily naps began to appear. This schedule of two naps daily persisted from the 25th to the 33rd week (Charts 7, 8 and 9). In general the morning nap was longer than the afternoon nap.

Between the 33rd and 34th week (Chart 9) there was a shift to a long afternoon "nap". Gradually the morning nap was eliminated and the baby remained awake all morning and took a fairly long afternoon nap. The beginning of this is indicated in Chart 9 between the 34th and 35th week.

In general the more isolated restless days and nights noted in this boy's Behavior Day Charts could be correlated with "teething", immunizations, illnesses such as colds or diarrhea or unusual family events such as extensive excursions

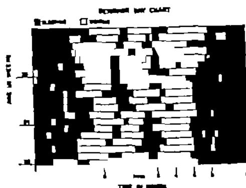


Fig 9

from the home or numerous visitors in the home.

Examples of this can be noted in the following weeks: 9th, a cold for 3 days; 10th, diarrhea on the 4th day; 12th, diarrhea on the 7th day; 26th, immunization on the 1st day; 27th, head cold for the entire week, 30th, immunization on the 6th day; and 35th, "teething"

The more lasting shifts in sleeping patterns could not be explained by observed events as noted above. In a general way they coincided with gross changes in the total development. For example, between 5 and 6 weeks a consistent smile could be elicited At this time some evidence of a diurnal cycle became apparent and the extreme restlessness of the preceding 2 weeks subsided. A bigger change came after 12 weeks when the baby could lie awake and amuse himself by cooing and striking at his cradle gym with his hands He began to play instead of crying when he awakened. At this time his sleep from 6 P.M to midnight became consistent and undisturbed After 20 weeks of age there was a major change in his posture during the daytime from the supine position to supported sitting and after 26 weeks he was sitting for prolonged periods with support He played happily in this position, reaching for objects and transferring them from hand to hand. By 32 weeks he could sit for prolonged periods unsupported. During this period his total sleep pattern, day and night, was better organized than at any other time during this study

After 32 weeks he began to become more mobile, changing from the seated to the prone position, and at 33 weeks he was crawling restlessly and getting himself into awkward positions. This mobility extended to a shift to the upright posture when at 34 weeks he began to pull himself to a standing position. This he enjoyed doing but he could not let himself down again and this made him fussy Coincidentally his day and night time sleep became somewhat irregular again. It also should be noted that the mother states he was teething during the 35th week.

Before a new organization of his sleeping patterns there often seemed to be a dissolution of the old one with a disorganized transition period. For example the 5th, 22nd, 25th and 34th weeks. It would appear that this boy went through recurrent periods of rather complete disorganization as he passed from one major developmental stage to another Gesell & Ilg (9) indicate that this is not uncommon throughout childhood.

Moore & U ko (17) reported that in the first 3 month illness caused only brief disturbances in sleep, but later in the year an illness could cause a prolonged period of sleep disturbances. They also noted in the first 3 months external stimuli such as sharing a room or other sources of noise did not seem to influence settling. After the fourth month external circumstances seemed

to play a more important part in disturbing the baby's sleep. They did not discuss disruptions from changes in total developmental patterns.

Of additional interest is the observation that this baby showed evidence of a 25 hour rather than a 24 hour cycle until about the 16th or 17th week of life. Comparable feedings or wakeful periods on successive days seem to occur about one hour earlier making a complete shift to the left on the charts in approximately 23 days. This is analogous to the case reported by Kleitman & Engelmann (15) with a 25 hour cycle and a similar daily shift in the opposite direction.

Comments

There is a striking similarity in the general evolution of the patterns of sleep and wakefulness in this case and the other single cases reported as well as in the larger group studies previously mentioned. Moore & Ucko (17) noted this unity within their own study and commented as follows: "More surprising than the number of deviants from the norm when (1) is considered, is the number who adhere to it in the face of difficulties."

Essentially the changes in the sleeping and wakefulness patterns from birth through at least the first eight months are not so much a decrease in the total amount of sleep but in the distribution of the periods of sleep and wakefulness. The neonatal infant has a significant amount of wakeful time in 24 hours, approximately 6–7 hours as is indicated in this case and in a study of the sleep of 75 newborn infant during the first few days of life (10). However this is broken up into many short periods and in this way appears to be negligible. Infants in the early weeks of life seem to be incapable of sustaining a long period of sleep or a very long period of wakefulness. As they mature they acquire the ability to do both. Thus, the short periods of sleep consolidate into larger ones, especially in the night, and the short periods of wakefulness consolidate into larger ones, especially in the daytime. This is well documented in the study of Kleitman & Engelmann (15).

The total amount of sleep per day at each age level noted in this report is somewhat greater than the averages found by Kleitman & Engelmann (15). This could be accounted for by the fact that in this instance only the mother judged when the baby was awake or asleep, whereas in Kleitman & Engelmann's study this was checked more carefully with actograms. Thus, the periods when the baby was awake and active but not active enough or fussy enough to attract a mother's attention would be noted by Kleitman's technique and the total sleeping time recorded would tend to be shorter. Nevertheless, this child shows the same general patterns of development as indicated by Kleitman & Engelmann (15).

The exact nature of the process of development of sleep patterns as described is unknown. Moore & Ucko (17) concluded that babies do not "learn" to sleep all night in the sense that this is something that can be actively taught them by their parents. What we have called the settling process, a form of learning at the level of biological adaptation, requires no consciously directed training by parents. There are indications that it is connected with fundamental physiological changes affecting the whole pattern of mental and physical activity.

Aserinsky & Kleitman (3) have studied eye movements and bodily movement of infant during sleep. They established

basic rhythmic cycling of motility of approximately 60 min between peaks of motility. They suggest that the duration of sleep is likely to be one or a multiple of these motility cycles. In other words, an infant is most likely to wake up during the period of the motility cycle when he is most active. If he does not wake up at this time, he is likely to sleep through another motility cycle. The origin of this basic cycling of motility is not known. They considered it to be of internal origin, relatively independent of environmental factors.

Jouvet (12) has found in cats rhythmic bursts of electrical activity in the reticular formation of the pons that is related to deep sleep. This activity can be detected in intact animals with chronically implanted electrodes when they are in deep sleep, but is obscured in light sleep. The chronic mesencephalic preparation cats, on the other hand, have only this type of sleep, as indicated by the recordings from electrodes in the reticular formation of the pons, electromyographic recordings of the neck muscles and respiratory changes. The rhythmic discharging that causes this type of primitive sleep in cats seems to be independent of the external milieu.

One might postulate that the newborn infant has rhythmically induced sleep of short duration that is independent of his external environment. Superimposed on this is some ability on the part of the infant to respond to external stimuli and to be aroused, especially between discharges, and then to maintain this arousal despite a sleep cycle or as Aserinsky & Kleitman (3) state, maintain sleep despite a motility cycle In any event, sustained arousal may be a function of higher central nervous system feedback to the reticular activating system (6) and sustained sleep may be partly dependent on the active suppression of the effectiveness of external sensory experiences or habituation (11 13 20). As the young infant s central nervous system develops, it is increasingly capable of sustaining arousal and of screening out some sensory experience in order to maintain prolonged sleep. Further studies will be necessary to elucidate this problem. Such complex behavior as sleep and wakefulness probably cannot be explained sufficiently by such a simple postulate.

Summary

A review and analysis of the sleep and wakefulness patterns of one boy from birth to 35 weeks of age has been presented. This boy's total sleeping time decreased gradually from 17.8 hours in the first week to 14.3 hours at 35 weeks The most remarkable changes were in the increase in the duration of the sleep and waking periods. The average longest sleep period for the first week was 4.4 hours and for the 29th week 11 7 hours. The frequency of wakeful periods dropped from an average of 7.5 in the first week to 4.2 in the 35th week. A diurnal sleep pattern is apparent in the Behavior Day Charts from the sixth week on and very definite after the 12th week. The sleep pattern noted in this case are essentially the same as reported in the literature cited Essentially all have noted predominantly a lenghthening of single sleep and wakef l periods with maturation in the first year rather than a marked reduction in the total amount of sleeping time

Possible neurophysiological explana tions for the changes in sleep patterns are discussed

References

1. Abramson, H. A. (Ed.): Problems of Consciousness; Transactions of the First Conference 1950, Josiah Macy J Foundation, 1951
2. Adrian E. D and Jasper, H. H. (Eds.): Brain Mechanism and Consciousness, Charles C. Thomas, Springfield, Illinois, 1954.
3. Aserinsky E. and Kleitman N A motility cycle in sleeping infants as manifested by ocular and gross bodily activity J Appl Physiol, 8 11 1955.
4. Buhler, C. and Hetzer, H.: Soziologische und psychologische Studien uber das erste Lebensjahr Jena, Gustav Fischer 1927
5. Debré, R. and Doumic A.: Le Sommeil de l'Enfant. Presses Universitaires de France, Paris, 1959
6. French, J D Hernandez-Peon R. and Livingston R. B Projections from cortex to cephalic brain stem (reticular formation) in monkey J Neurophysiol, 18 74, 1955.
7. Gesell, A. and Amatruda, C. S.: Embryology of Behavior Harper New York, 1945
8. Gesell, A. and Ilg F Feeding Behavior of Infants, Lippincott, Philadelphia, 1937
9. — Infant and Child in the Culture of Today Harper New York, 1943.
10. Hellbrügge, T Lange, J and Rutenfranz, J Schlafen und Wachen in der kindlichen Entwicklung Beiheft zum Arch fur Kinderheilkunde No. 39 Ferdinand Enke, Stuttgart, 1959
11. Jasper, H. H.: (Chapt. 15). Recent advances in our understanding of the ascending activity of the reticular system. In the Henry Ford Hospital Symposium, Reticular Formation and the Brain, Detroit, 1957
12. Jouvet M., Michel, F and Courjon, J Sur un stade d'activité électrique cérébrale rapide au cours d sommeil physiologique C R Soc Biol 153 1959, 1959.
13. Jouvet M. and Michel, F Aspects électroencéphalographiques de l'habituation de la réaction d'éveil. J Physiol (Par), 51: 489, 1959
14. Kleitman, N Sleep and Wakefulness, as Alternating Phases in the Cycle of Existence. Univ of Chicago Press, Chicago, 1939
15. Kleitman N and Engelmann T. G Sleep characteristics of infants. J Appl Physiol, 6 269 1953
16. Magoun H. W The Waking Brain, Springfield, Charles C. Thomas, Illinois, 1958.
17. Moore, T and Ucko L. D Night waking in early infancy Arch Dis Child, 32: 333, 1957
18. Muller F J Development of the diurnal temperature and motility pattern in a baby Amer J Physiol 118 462, 1938.
19. Parmelee, A. H., J Schulz, H. R. and Disbrow M. A.: Sleep patterns of the newborn; To be published in J Pediat, 57 1961.
20. Sharpless, S and Jasper, H. H.: Habituation of the arousal reaction. Brain, 79 655, 1956.
21. Simsarian F P and McLendon, P A: Further records of the self-demand schedule in infant feeding J Pediat, 27 109 1945.

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CASE REPORT

Icterus and Xanthomatosis

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Xanthomatous changes in the skin are rare in children and comparatively little has been published on the subject from pediatric quarters. Crocker (5) and Moriquand (14) independently deal with xanthomatosis in children in their reviews, and it is apparent that the etiologic and pathogenetic possibilities are many. Disturbances of the liver and bile ducts may cause xanthomatosis in children. No conclusive proof that acute virus hepatitis has been the causative factor in xanthomatosis has yet been reported in medical literature. In fact, Thannhauser (17) categorically maintains that the two do not occur together. On the other hand, Magnusson, Josephsen & Tungland (13) in 1956 reported a case of xanthomatosis associated with icterus in a 7 year old boy. They considered an acute viral hepatitis to be the most likely etiology. We have had occasion to study an almost identical case in the Pediatric Department, Oslo University Hospital, and thought it worthy of notice.

Case report

The patient is a boy born April 25, 1954, who has previously been in good health. He comes from a healthy family none of whom have cutaneous xanthomas. The parents and two other siblings are living and in good health. The pregnancy, delivery and neonatal period were uneventful. No nutritional problems. Psychomotor development normal.

The first manifestation of the present disease occurred at the age of two years. The boy became listless, irritable, somewhat feverish and would not eat. After about a week, dark urine, clay-coloured stools and jaundiced skin were observed. Information was obtained that no other case of jaundice had occurred in the district at that time. As for the possibility of inoculation hepatitis, it was reported that the patient had had his last injection of polio vaccine in November 1957.

During the following 4 to 5 weeks, the icterus gradually became pronounced, and the patient developed pruritus. He stayed at home partly confined to bed, and was given a light diet but no other treatment. In the beginning of May he was admitted to the Department for Epidemic Diseases, Central Hospital, Trondheim. Marked jaundice, positive Gmelin, and negative Schlesinger reaction in the urine were demonstrated. The patient was discharged from the hospital after 8 days with the diagnosis of acute hepatitis. His condition remained the same. The jaundice persisted without change for about 8 weeks after discharge from the hospital, and the boy continued to be listless and uncomfortable from the itching. From the beginning of July the patient improved,



Fig 1

the icterus decreased and the urine became lighter At the same time, however an eruption of yellowish white nodules on hands and feet was observed When the patient was examined by the referring pediatrician at the end of July a moderate icterus was still present and there was a general eruption of xanthomas in addition. The patient was therefore referred to the Pediatric Department Oslo University Hospital

On admission, Aug. 12, 1955, the boy was two and a half years old. He was thin, somewhat pale, afebrile and not icteric. The n was spotted with small yellowish white particulary along the lines of palms and soles. Efflorescences of the oral mucosa, specially on the gingivae, were also demonstrated (Fig 1 and 2) The liver was considerably enlarged, extending to the umbilical level, and was slightly tender The surface was smooth and firm. Splenomegaly was not demonstrable. N other pathologic findings were brought to light by the clinical examination.

Laboratory findings: Routine chemical and microscopic examination of the urine revealed nothing abnormal. The serum was *markedly turbid*. Hemoglobin: 75 %. Sedimentation rate: 84 mm per hour Leucocytes: 5100 mm distribution normal. Erythrocytes: 3.84 millions per mm³ Bilirubin (total) 0.6 mg%. Alkaline phosphatase: 25.7 Bodansky units. Takata's reaction negative Serum proteins: Albumin 3.7 g ...



Fig 2.

Globulin 4.6 g%. Alfa 2 globulin 1.3 g%. Beta globulin 1.8 g%. Gamma globulin 1.2 g%. Total lipids: 5310 mg% (normally 400–700). Total cholesterol: 1742 mg% (Table 1).

Roentgenographic examination of the skeleton revealed considerable osteoporosis. A cholangiogram disclosed normal gall bladder and good excretion f contrast. Biopsy of the skin: Xanthomatosis.

The patient was placed on a diet low in cholesterol and given meticorten with the dosage of 5 mg four times a day. In the course of 12 weeks, this was reduced to 2.5 mg twice a day. Subsequently the xanthomatosis became less marked, the liver less enlarged and the biochemical changes showed steady improvment (Table 1). He was discharged from the hospital on November 3, 1958 and instructed to follow a diet low in cholesterol and continue to take meticorten 2.5 mg twice daily.

The patient was later admitted in F bruary 1959 and September 1959 for reexamination. For practical reasons, it had not been possible to continue his low cholesterol diet, and the meticorten medication was discontinued in March, 1959. He had enjoyed excellent health all the time. On the final examination, the xanthomas were considerably reduced, had complet ly disappeared from the face and mucous membranes, but were still present in both palms and on buttocks. The liver was no longer palpable. Laboratory findings of significance are shown in Table 1

Discussion

A simultaneous occurrence of icterus and xanthomatosis raises etiologic pathogenetic and diagnostic considerations which have long been a controversial subject. Depending on the author's view as to the etiology and pathogenesis opposite conclusions have been drawn at dif ferent times, particularly as to whether the liver and bile duct disturbance or the xanthomatous is the primary cause of the condition

On the basis of recent studies and the findings in this particular case the rela tion between icterus and xanthomatosis in our patient will be discussed below

The diagnosis of acute viral hepatitis can usually be made with reasonable

TABLE 1 *Laboratory data during the course of the disease.*

	1958 Aug. 15	1958 Aug 21	1958 Sept. 9	1958 Oct. 23	1959 Febr 26	1959 Sept. 16
Total lipids	5310 mg %	4343 mg	1890 mg*	1123 mg %	860 mg %	710 mg*
Neutral fat		1317 mg	706 mg %			
Free fatty acids		100 mg*	200 mg*	190 mg	} 340 mg %	290 mg
Total cholesterol	1712 mg*	1248 mg	624 mg %	230 mg	46 mg %	1 8 mg
Phospholipids		1690 mg	348 mg	320 mg	234 mg	235 mg
Alkaline phosphatase (Bodansky)	23.7		12.7		5.1	9 3
Thymol	0.15		0.13	0.16	0.03	0.06
Takata	Negative		Negative			Negative
Prothrombin-proconvertin time in of normal	120			125	83	115
Bilirubin	0.6 mg				0.8 mg	
Total serum proteins	8.3 g		7.3 g	7.2 g*	6.9 g	7 1 g
Sedimentation rate	84 mm per hour		12 mm per hour	23 mm per hour		11 mm per hour

certainty from the clinical data and simple laboratory examinations. It is well known however that the disease can appear in different forms. One of there is the so-called cholangitic form, characterized by protracted icterus and pruritus and laboratory findings that point to an obstructive disturbance rather than to parenchymatous injury The patients recover slowly with complete recovery usually occurring within a year

Our patient presented just this picture. Icterus and pruritus persisted for 4 months. The alkaline phosphatase was increased, electrophoresis of the serum proteins revealed findings that indicated an obstructive disturbance, while routine tests for a parenchymatous injury revealed nothing abnormal. Apart from the occurrence of xanthomatosis the convalescence was protracted and the liver did not return to normal size for about a year There were no reports of other cases of jaundice in the patient s home district, but the possibility of hepatitis resulting from oculation is present, as the patient was ted against poliomyelitis 4 months before the onset of the disease

This type of hepatitis does not seem to be rare Ellakim & Rachmilewitz (7) present a study of 104 cases of acute hepatitis. Nearly one fifth of the patients showed the picture of the cholangitic type of illness. In these the condition was practically indistinguishable from that of obstructive jaundice Liver biopsy is a most helpful diagnostic measure (4) but unfortunately we do not have this conclusive proof in our patient The course of the illness, however makes the diagnosis viral hepatitis of cholangitic type highly probable.

The main point of interest is the second stage of the disease, the development of the xanthomatosis, and the relationship between this and a hepatitis of cholangitic type. As mentioned in the introduction, it is well known that many liver and bile duct disturbances can engender secondary xanthomatosis. This phenomenon has been reported in liver glycogenosis (19), toxic hepatitis (6) congenital dysplasia of the interlobar bile ducts (2, 5 8 11) protracted bile stasis caused by extrahepatic occlusion of varied etiology (3) and in primary biliary cirrhosis (11 15, 16). These conditions have the following common traits. a more or less chronic bile stasis serum lipids which are demonstrably elevated, and liver function tests which remain normal for a long time. Ahrens & Kunkel (1) found that patients with total lipid serum values of more than 2000 mg %, lasting for more than 6 months. always developed xanthomatosis, whereas xanthomatosis did not develop in those with serum lipid values below 1200 mg % no matter what the duration

Our patient presented all of these characteristics. The clinical evaluation and, even more, the course of the patient s illness do not however point to the presence of any of the above mentioned liver and bile duct disturbances. During the first part of the first hospitalization the picture presented came closest to that of xanthomatous biliary cirrhosis. This somewhat nebulous syndrome today interpreted as a primary liver disease of various etiology (3, 6 11 16) is a chronic condition with remittent attacks of jaundice and a poor long term prognosis This disease can thus be ruled out as a diagnostic possibility in our case

Primary biliary cirrhosis can, as mentioned above, give rise to secondary xanthomatosis. In this connection, it should be noted that primary biliary cirrhosis may develop as a consequence of acute cholangitic hepatitis (18). It seems likely that the same pathological mechanism can be at work in both stages. Therefore it is logical to assume that xanthomatosis can evolve from an acute as well as a chronic phase provided the prerequisites for xanthoma formation are present.

It is interesting to note that in all previously reported cases with secondary xanthomatosis caused by liver and bile duct disturbances the plasma has always been clear. The rise in the serum lipids was entirely due to an increase of cholesterol and phospholipids while neutral fat concentration was normal. In our patient, the plasma was turbid, owing to a marked increase in the neutral fat concentration (Table 1). This finding may suggest the possibility of a particular primary disturbance in the lipid metabolism, i.e. idiopathic familial hyperlipemia. The plasma in this condition is highly lipemic all lipid fractions are considerably elevated and xanthomatous deposits in the main biliary ducts may lead to bile stasis with jaundice. The clinical picture is then indistinguishable from secondary xanthomatosis caused by primary liver and bile duct disturbances (16). These are precisely the differential diagnostic possibilities emphasized by Magnusson and his coworkers (9 13). In the present case the possibility of primary xanthomatosis seems completely unlikely. There is nothing to support a hereditary predisposition in the family history, the xanthomatous skin changes did not occur until the icterus had practically subsided, and roentgen contrast examination of the bile ducts revealed good excretion. It seems unthinkable that resorbtion of primary xanthomatous deposits in the bile ducts should take place in the presence of severe cutaneous xanthomatosis and while the serum lipids were markedly elevated. The most likely conclusion seems to be that the patient reported here has had a primary liver disease, probably a hepatitis of cholangitic type with development of secondary xanthomatosis.

Summary

The clinical course of a two and a half year old boy with icterus, hepatomegaly, xanthomatosis and marked elevation of the serum lipids is described. In the process of this disease these clinical and biochemical signs slowly disappeared and the patient seems to have regained complete health. The diagnostic problems are discussed. It is concluded that the patient suffered from a primary liver disease most likely viral hepatitis of the cholangitic type with development of secondary xanthomatosis.

References

1. A[illegible], E. H. J. and K[illegible], H. G. Relationship between serum lipids and skin xanthomas in [illegible] patients with primary biliary cirrhosis. J Clin Invest, 28 1949, 1 49
2. A[illegible], E. H., H[illegible], R. C. and MacMahon, H. E. Atresia of the intrahepatic bile ducts. Pediatrics, 8 628, 1951
3. Ahrens, E. H. J., P[illegible], H. G., Kunkel, W. J., E[illegible], W. J. and Bloomberg, B. H. Primary biliary cirrhosis. Medicine 29 299 1950

4 CALL, E. A. and BRAUNSTEIN H.: Hepatitis with manifestations simulating bile duct obstruction. *Amer J Clin Path* *25* 1153, 1955.
5. CROCKER, A. C.: Skin xanthomas in childhood. *Pediatrics*, *8* 573, 1951
6. DUBIN I. N Cholestatic hepatitis. *Bull NY Acad Med*, *32* 306, 1956.
7 ELIAKIM, M. and RACHMILEWITZ, M.: Cholangitic manifestations in virus hepatitis. *Gastroenterology* *31* 369 1956.
8. HAAS, L. and DOBBS, R. H.: Congenital absence of the intrahepatic bile ducts. *Arch Dis Child*, *33*. 396, 1958.
9 JOSEPHSEN J O Xanthomatose og levergallevejslidelser *Nord Med*, *55*. 77 1956.
10 MACMAHON H. E.: Die biliäre Xanthomatose bei Erwachsenen und Kindern. *Virchow Arch Path Anat* *321* 664 1952.
11 MACMAHON H. E. and THANNHAUSER, S. J Xanthomatous biliary cirrhosis (a clinical syndrome). *Ann Int Med*, *30* 121 1949
12. — Congenital dysplasia of the interlobular bile ducts with extensive skin xanthomata: Congenital acholangic biliary cirrhosis. *Gastroenterology* *21* 488 1952.
13 MAGNUSSON R., JOSEPHSEN J O. and TUFOLAND B.: Icterus og xanthomatose *Nord Med*, *55* 83, 1956.
14 MOURIQUAND CL.: Les xanthomes cutanés chez l'enfant et le nourrisson. *Pédiatrie* *3* 163, 1957
15. NEZELOF R. J Ch., ROBINEAU, M. and JOS, J.-C.: Ictère cholostatique chronique par cholangiolite et péricholangiolite (syndrome de MacMahon) chez un enfant de 18 mois. *Ann Pediat (Par)*, *35-44*. 3123, 1959
16 SHAY H. and HARRIS, C.: Changing concepts of "xanthomatous biliary cirrhosis *Amer J Med Sc* *223*. 286, 1952.
17 THANNHAUSER, S. J Lehrbuch des Stoffwechsels und der Stoffwechselkrankheiten. Georg Thieme Verlag, Stuttgart. 1957
18. WATSON J C. and HOFFBAUER, F W The problem of prolonged hepatitis with particular reference t the cholangiolitic type and the development of cholangiolitic cirrhosis of the liver *Ann Int Med*, *25* 195, 1946.
19 ZAKOU S J OYAMAD A. and ROSENTHAL, I. H.: Eruptive xanthoma and hyperlipemia in glycogen storage disease *Arch Dermat Syph* *67* 146 1953.

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CASE REPORT

Hereditary Renal Dysplasia and Blindness

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Hereditary progressive renal disease was first described by several authors around 1900. Mitchell (12) reviewed the literature in 1930. Since then a similar clinical picture has been reported combined often with abnormalities in other organs.

Ryerbach (13) described a neurogenic deafness. Hawkins & Smith (7) reported various skeletal and skin abnormalities in 36 cases. These belonged to 4 generations of the same family. Congenital cataract was present in 3 of their cases. Sohar (15) described 10 cases among whom 2 had *spherophakia* and 2 had congenital cataract. Goldbloom (6) reported 3 siblings aged 5, 10 and 18 years, all of whom died with uremia. Two had reduced hearing, and one of these also an anterior subcapsular cataract. In no case has histological examination of the eyes been performed.

In the few autopsied cases the renal lesion was found to be pyelonephritis. The pathogenesis, however was unclear until Marshall (11) and Ericsson & Ivemark (3, 4). Ivemark, Oldfeldt & Zetterström (8) observed that pyelonephritis may develop on the basis of renal dysplasia.

The present report deals with 2 blind siblings who died with renal failure at 8 and 9 years of age. In one case a postmortem examination including the eyes was performed. The parents are unrelated. Several members of the fathers family have died of "stroke" at the age of 60–70 years. Three of the mother's siblings have died, one from diabetes, one from multiple sclerosis and one from lymphosarcoma. No other hereditary disease or abnormalities are known in the family.

Case Reports

Case 1 T.A., male, born May 29 1939. The pregnancy was uncomplicated and delivery was uneventful. Birth weight 4350 g. When he was 3 months old the parents noticed that his eyes did not follow light or objects, but he reacted to strong light stimulation by closing his eyes. The psychomotoric development was markedly retarded. At age 2 years he was admitted t the Eye Department and Pediatric Clinic of Rikshospitalet Oslo.

His musculature was hypotonic and he showed both mental and motor retardation. The eyes were slightly sunken in, there was nystagmus, and he did not focus on any object. The pupils reacted sluggishly to light. Ophthalmoscopy revealed normal eyegrounds. The following years an increasing mental retardation occurred. He could only utter a few words; he was confused and

agitated, had a poor appetite and vomited frequently He was cared for in a home for mentally retarded children.

At 7 and 7½ years old he was again seen in the Pediatric Clinic. Weight 14.6 kg height 107 cm, blood pressure 100/60. The skin was pale and dry with petecchiae. Examination of the eyegrounds was normal as was his hearing. This condition grew increasingly worse. Clonic seizures occurring particularly at night began and at 8 years of age he died in a local hospital following a short period of vomiting, diarrhea and repeated convulsions.

Laboratory findings Urinalyses were normal when he was 2 years old At 7 and ½ years proteinuria was repeatedly present (Esbach was ½ %) and specific gravity was invariably below 1 010 Benzidine reaction was negative, and microscopy showed a few white blood cells and granular casts.

Blood At 2 years of age the Hb. was 82% and sedimentation rate 2 mm. At 7 years Hb. was 50%, erythrocytes 2.36 mill/mm leucocytes 9.300 with normal differential count. Bone marrow biopsy showed scarce erythropoiesis. Serum iron was 92 gamma %. Bleeding time, coagulation time and prothrombin time and clot retraction were normal. Pneumoencephalography was unsuccessful. ECG and X ray of the heart were normal.

Case 2 T.A. female born November 7 1950 following an uncomplicated pregnancy and delivery Birth weight was 2830 g. No neonatal complications occurred Her growth and motor development were normal. She was said to have fixed on objects at 2–3 months of age However at 5½ months of age she was admitted to the Pediatric Clinic, Rikshospitalet because of reduced vision. She did not follow any object with her eyes. The eyegrounds and the remaining examination were normal.

In the following years she continued to develop normally e cept for her visual defect At 7 years complai ts of weakness, abdominal pains and nausea began. She wa fo nd to be anemic and was readmitted to the Pediatric Clinic Physical examinat n ak l a pale nd thin girl wh w ighed 24 kg and measured 122 cm. Some light perception was still preserved. The pupils were dilated and reacted sluggishly to light. Ophthalmoscopy showed normal optic disc. The macular regions ppeared normal, the vessels showed no pathological changes and in the periphery a diffuse pigmentation as seen. The refracting media were clear Her hearing was normal. Because of the anemia she received blood transfusions, and cortisone was tried without effect.

Her condition remained unchanged for 2 years. At 9 years of age she became increasingly fatigued with headache and vomiting her gait was spastic and she had periodic clonic jerks. She became dyspnoeic and stuporous and was readmitted to the Pediatric Clinic. Her skin was extremely pale with multiple petechiae and ecchymoses. Blood pressure was 170/100 Blood and saline transfusions were given without effect, and she died the next day following repeated convulsions.

Laboratory investigations At 5 months of age her blood and bone marrow were normal.

Seven years old: *Urinalyses*. The protein reactions were positive and the specific gravity was below 1.010, even after a concentration test. Microscopy revealed 8–10 white blood cells per visual field as well as scattered granular casts. Urine cultures were sterile.

Blood. Hb was 58%, erythrocytes 2.70 mill/mm³ reticulocytes 3%, thrombocytes 120,000–210 000 mm³ leucocytes 12,200 mm³ with a shift to the left of the differential cell count. Sedimentation rate was 25 mm. The bone marrow was normal. Serum iron 85 gamma %, creatinine in serum was 3.5 mg% urea 132 mg%. CO_2-combining power 34.7 vol%, calcium 9.8 mg% phosphorus 5.4 mg% and alkaline phosphatase 14 units (Bosch) The dye test was negative and the antistreptolysin titer 700. Electrophoresis showed an increase of alpha₂-globulins but was otherwise normal. X-ray of the skeleton and retrograde pyelography were negative. Electroencephalogram showed no definite pathological changes.

Nine years old Hb. was 4 sedimenta



Fig. 1 Section from the kidney showing loss of convoluted tubules. Groups of dilated tubules are seen. Only a few glomeruli are preserved. Lymphocytic infiltrations. Increased connective tissue. Hematoxylin-eosin. 80.



tion rate 20 mm, urea 424 mg%, CO_2-combining power 10.7 vol% and chlorides 103 mEq.

Autopsy. The pertinent findings were: Small amounts of serous fluid in the pleural cavities (ca. 20 ml) in the pericardial cavity (5 ml) and in the peritoneal space (100 ml). The lungs were edematous and hemorrhagic. The heart was slightly enlarged, weighing 170 g, and there was some hypertrophy of the left ventricular wall.

The kidneys weighed 28 g and 27 g. They appeared shrunken, and their surface was slightly granular. The cut surface had a yellowish tinge and the cortical substance varied in width from 1–3 mm. Pelves, ureteres and bladder were normal.

Microscopic examination of the kidneys (Fig. 1) revealed distorted cortical structures, primarily due to atrophy of the tubules. Only a few scattered groups of dilated



Fig. 3 Brain stem with edema and small hemorrhages. Fibrinoid material in the perivascular spaces and deposited in the surrounding brain tissue. P.T.A.H. 80 ×.

primitive glomeruli were also found. The parietal layer was coated by columnar or cuboidal epithelial cells. The tufts were bizarre and made up by cuboidal, dark staining epithelial cells and deposition of mineral salts occurred.

Gross examination of the brain showed ly some flattening of the gyri. The weight 1400 g. After 14 days fixation in 10 % formalin the brain was cut. A few pin prick sized hemorrhagic spot were present in pons and tegmentum. Microscopically these areas showed an edematous tissue. There was some swelling and proliferation of the endothelial cells as well as of the adventitial connective tissue. The vessels were surrounded by a mostly cell free slightly eosinophilic staining substance bordered by a dense fibrinoid material deposited in the brain tissue (Fig. 3). By Mallory's connective tissue staining the material stained brightly red or more orange. It was PAS positive and stained dark purple-blue by P.T.A.H. A few perivascular hemorrhages and gliosis were also found. The optic pathways, such the superior colliculi, the geniculate bodies, the optic radiation and area striata were normal.

The posterior parts of the eyes were removed through the orbital roof with the optic nerves. They were fixed in Bleuin fluid and embedded in paraffin.

The two eyes showed nearly identical microscopical pictures. The sclerae and choroidal layers were normal. The pigment epithelium was preserved, but the pigment was rather scarce. There was some scattering of pigmented cells in the choroidal layers. The retina was separated from the pigment epithelium. There was a marked reduction of cells. In the macular regions one single layer of rather large cells of an epithelial appearance replaced the outer granular layer (Fig. 4), probably representing the ependymal cell layer from which the rod and cone cells normally develop. The external limiting membrane could be seen separating the cell bodies from their cone shaped extensions. No rod cells could be identified. In more peripheral parts of the retina the outer granular layer contained only few irregularly distributed cells (Fig. 5), and few pigment-laden cells were scattered among them, sometimes gathered around small vessels. The plexiform layers as well as the

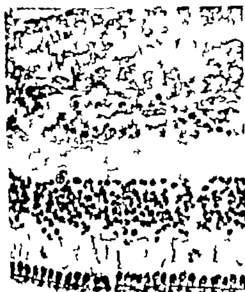


Fig 4. Macular region of the left eye The outer granular layer is represented by one single layer of cells. The external limiting membrane is preserved. The inner granular layer is fairly well developed, and a few ganglion cells are seen. P T.A.H. 220.

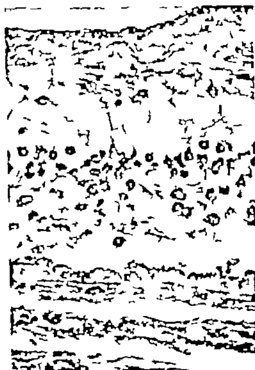


Fig 5. The retina, pigment epithelium and choroidal layers in peripheral part of the right ey The various retinal layers are severely educed only the fiber layer is thickened. P T.A.H 460

inner granular layer were markedly atrophic, but not t the same degree as the outer granular layer Some ganglion cells were preserved. The nerve f ber layer was enlarged by growth of glial fibers, and con tained well preserved vessels f the usual size and shape The optic nerv was normal.

Discussion

In the previously reported cases of hereditary nephropathy combined with deafness or less commonly ocula disturbances, the true nature f the renal lesions seems obscure In the few cases coming t autopsy the histological examination has revealed wide-spread pyelonephritis. Goldbloom *et al.* (6) emphasized that the pathological findings probably represented end stages completely masking the under lying primary renal lesion. In an attempt to explain the peculiar susceptibility of these individual to pyelonephritis these authors suggested m ltifocal hypoplasia or malformations as the possible primary cause In the absence of an tomic abnormalities an inherited enzymatic defect was considered. Studies by Marshall (11) and more recently by Ericsson & Ivemark (3 4) provide evidence for the assumption that patients with dysplastic kidneys are highly susceptible to pyelonephritis

In our Case 2 wide-spread chronic pyelonephritis with almost complete distortion of the renal parenchyma was evident. Primitive ducts or cartilage held to be indisputable evidence of dysplasia were not seen. However other structures, supporting the diagnosis of dysplasia as reported by Ericsson & Ivemark (3 4) were common in the specimens examined. These structures were ductules lined by cuboidal epithelium and surrounded by distinct rings of concentric connective tissue bands. Formations apparently representing primitive glomeruli were also encountered. Ericsson & Ivemark (4) were able to show that such ductules originated in the medulla and ended blindly beneath the renal capsule without joining uriniferous tubules. The primitive or abnormal glomeruli were isolated or connected with atrophic dilated proximal tubules with no outlet In their opinion, a kidney should be regarded as dysplastic when such ductules are found The primary lesion in the pre case is therefore believed to be renal lasia.

The degenerative changes of the vessel walls in the brain stem are believed due to the uremia The vascular changes are of the same kind as those found in the kidneys. The perivascular transudate indicates a disturbed blood brain barrier causing edema and deposit of an albuminous material in the brain substance This apparently resulted in a glial reaction around the vessels. The location of uremic changes in the brain stem is not unusual (*) and the wide-spread distribution in the pons and reticular substance of the brain stem accounts well for the convulsions, coma and death.

Congenital blindness has been the subject of recent large scale investigation. Alström & Olson (1) found that 10% of the blind children in Sweden from 1897 to 1948 belonged to the group classified heredoretinopathia congenitalis. This group included various clinical diagnoses such as retinochorioiditis, amblyopia congenita and atrophy of the optic nerve. The ophtalmoscopic picture may be normal in early cases, but more often dusty pigmentation was found which increased with age ERG was negative in most of the cases. Cataract and keratoconus were present, increasing in frequency with age. No increased frequency of mental deficiency compared to the rest of the population as found. Genetic studies showed a monohybrid recessive autosomal heredity

Schappert-Kimmijser et al. (14) studied 227 cases of amaurosis congenita in the Netherlands, and their descriptions are much in accordance with those of Alström & Olson. ERG could not be separated from those of retinitis pigmentosa. They also found an autosomal recessive heredity

Sorsby & Williams (16) described 9 patients with retinal aplasia in 2 families. Several also had mental retardation. These authors separated the condition from retinitis pigmentosa on the basis of the different clinical record, but suggested that the aplasias were related to retinitis pigmentosa, possibly caused by a more severe developmental deficiency They referred to Keeler's investigations (9 10) in the house mouse Based on histological examinations of the eyes and electromotive force generated through stimulation by light Keeler found mice with hereditary blindness mainly due to a reduction of the rod cells. The reduction was present in varying degrees, and he found that the pigment epithelium sometimes broke through the external limiting membrane and scattered pigmented cells were found between the cells in the outer layers f retina.

Histological examination in human cases of retinal aplasia has not been found in the literature The histological picture in the

present case is very similar to the description by Keeler (9 10) in mice, and it has also features common with retinitis pig mentosa. In our case the displaced pigment was rather scarce. Early cases of this disease have however been described as retinitis pigmentosa sine pigment (17) Our impression that the degeneration starts in the outer granular layer and spreads inwards, is also in accordance with Wolffs descriptions of retinitis pig mentosa. As to Keelers descriptions of the rodless mice the present case is very similar to his most advanced aplasias with depletion of rods and one layer of cones left.

The present case seems to display two different congenital defects. Both dysplastic kidneys and retinal dysplasias have been described as genetically determined abnormalities. The dysplastic kidney has been found combined with several other anomalies, most often with neurogenic deafness. The retinal dysplasias sometimes were combined with mental deficiency Whether the cataract described in cases of renal dysplasia might have been combined with retinal changes is not known It should, however be remembered that both cataract and keratoconus are frequently combined with retinal aplasia. Alström & Olson (1) concluded that the gene carrying the anomaly influenced both retina lens and cornea.

To the authors knowledge the occur rence of hereditary renal disease and congenital blindness has not been reported previously The histopathological find ings in one of our cases indicating renal dysplasia and retinal aplasia seem to present an unusual combination of two geneti cally determined abnormalities.

Summary

Two siblings, a boy and a girl, with congenital blindness died at 8 and 9 years of age from renal failure Autopsy was performed in one case. The kidneys showed evidence of dysplasia and of pyelonephri tis. Earlier suggestions that renal dysplasia might be the primary cause in cases of chronic pyelonephritis in children are supported. Examination of the eyes indicated a congential defect, retinal aplasia.

References

1 Alström, C. H. and Olson O Heredo-retinopathia congenitalis. *Hereditas*, 43, 1957 p 178.
2. Bodcorter, G and Eriksson, F Handbuch der speziellen pathologischen Anatomie und Histologie. XIII, B. 2. 1958, p. 1419. Springer Verlag, Berlin
3. Ericsson N O and Ivemark, B. L: Renal dysplasia and pyelonephritis in infants and children I. *Arch Path* 66 255, 1958.
4. — Renal dysplasia and pyelonephritis in infants and children. II *Arch Path* 66 264 1958.
5. Fraser Th: T tilfælde hereditær familiær kongenital (?) aefect. *Norsk Mag Lægev* 65 803 1904
6. Goldbloom, R. B Fraser, F C W von D Aronovitch, M and Wiglesworth F W Hereditary renal disease associated with nerve deafness and ocular lesions. *Pediatrics*, 20 421 1957
7 Hawkins, C. F and Smith, O E: Renal dysplasia in family with multiple hereditary abnormalities including fibes borne. *Lancet*, *1* 803, 1950.
8. Ivemark, B. L, Oldfelt V and Zetterström, R: Familial dysplasia of kidneys, liver and pancreas. A probably genetically determined syndrome *Acta Paediat*, *48* 1 1959
9 Keeler, C. E: Rodless retina, an ophtalmic mutation in the house mouse, mus musculus. *J Exp Zool*, *46* 355, 1927
10 Keeler, C. E: Normal and rodless retinae of the house mouse with respect to the electromotive force generated through

stimulation by light. *Proc Nat Acad Sc*, *14* 477 1928.
11 MARSHALL, A. G The persistence of foetal structures in pyelonephritic kidneys. *Brit J Surg* *41* 38, 1953.
12. MITCHELL, A. G Nephrosclerosis (Chronic interstitial nephritis) in childhood. *Amer J Dis Child*, *40* 101 1930.
13 RYKENBACH, G. C. and BUTLER, A.: Congenital hereditary hematuria. *New Engl J Med* *251* 377 1954
14. SCHAPPERT KIMMIJSER, J HENKES, H. E. and VAN DEN BOSCH, J Amaurosis congenita. *Arch Ophtal*, *61* 211 1959.
15. SOHAR, E.: A heredo-familial syndrome characterized by renal disease, inner ear deafness and ocular changes. *Harefuah*, *47* 161 1954. Cit. Goldbloom et al., *Pediatrics*, *20* 421 1957
16. SORSBY A. and WILLIAMS, C. E.: Retinal aplasia as clinical entity *Brit Med J* *169* 293, 1960.
17 WOLFF E.: A Pathology of the Eye. p. 163. Lewis and Co LTD London 1951

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PROGRESS IN PEDIATRICS

Polycystic Kidneys in Newborns, Infants and Children

A Clinical and Pathological Study

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Introduction

Cystic kidneys occur in adults as well as in children of all ages. While the adult variety of the disorder usually is inherited dominantly it may also occur without manifest heredity.

Cystic kidneys in children of various ages have been the subject of very divergent opinions, both as regards their relationship with the adult form and with respect to their heredity. Most authors still treat cystic kidneys as a disease entity where the degree of parenchymal destruction is the sole factor determining the patient's chances of surviving.

As early as 1902, Küster showed that the age distribution of 239 subjects with the disorder had two peaks: one at the time of birth (59 stillborn babies) and another between the ages of 30 to 60 years (Fig. 1). Later other authors have confirmed this distribution (9, 23). The consensus of all workers who in recent years have studied the age distribution of cystic kidney cases is that the disorder is encountered in children of all ages, albeit less frequently in infants and young children. Yet no preponderance is found among newborns and infants in any of the published family trees showing the inheritances of the dominant adult form, actually, as far as we know, it has not been diagnosed in any child less than 2 years of age in these families and some workers believe that cystic kidneys in newborns and children are recessively inherited (6, 23).

Studying cystic kidneys in newborns (2 cases) on the one hand, and in older children and adults on the other, Lambert (18) demonstrated that the cystic kidneys in the two groups differed histologically. The existence of such a difference has subsequently been verified (4). Continued research on the subject has revealed not only that there exist two forms of the condition—the neonatal and the adult—but also that the former occurs in two or more histologically discrete varieties (22, 25, 30).

Many problems posed by newborns and infants with cystic kidneys—their clinical manifestations, heredity and prognosis as well as the question of their relationship to cystic kidneys in older children and adults—will hardly be solved unless

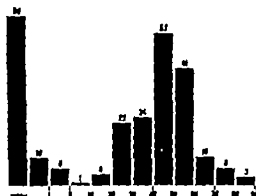


Fig. 1 Age distribution according to Köster



Fig. 2. Age distribution in present material. ■ Group I □ Group II, ▯ Group III-IV

one finds means of morphologically distinguishing the different types. Unfortunately most monographs on cystic kidneys have in common the weakness that the material has been imperfectly analyzed from histological points of view and hence clinical and genetic peculiarities are obscured or difficult to interpret. Dalgaard (6) for example drew conclusions about cystic kidneys in newborns and children despite the fact that in his series of 40 cases the kidneys were not examined microscopically

Material (Including the Criteria of Diagnosis)

According to our concept cystic kidneys are characterized by bilateral diffuse scattering in the parenchyma of a large number of cysts of varying sizes. The microscopical appearances range from those where the cysts are entirely dominant and look like the meshes in a coarse network supplanting the normal renal parenchyma, to those where the cysts are small and interspersed by normal-looking parenchyma. Grossly the kidneys may exhibit the usual kidney-shape or may be greatly deformed; they may be anything from hypoplastic to enormously hyperplastic. We have excluded from the series all cases of unilateral cystic kidney where the contralateral organ was normal, as well as all cases of circumscribed unilocular or multilocular cystic formations.

Potter (22) described familial occurrence of large spongy polycystic kidneys in newborns. The familial occurrence of this type was also observed by one of us (20). This prompted the idea of analyzing the clinical



Fig. 3. Polycystic kidneys in situ. Group I.



Fig. 4. Polycystic kidney Group I. 0.8.

manifestations and heredity of such neonatal cystic kidneys and led to the collection f a 10 year series from the whole of Sweden.

After perusal f the Annual Reports for the decade 1945–1954 from all Swedish hospi tals, relevant extracts from the case records were collected regarding 27 cases which complied with our criteria. T these cases ere added 14 similar ones observed from 1945 through 1958 in Gothenburg (Table 2). In 26 of these cases t proved feasible to carry out a thorough histological examination. In addition the xistence f cystic kidneys in living hild (7 B sibling of child 7 A) was established by clinical diagnosis.

Fig 2 shows the age classification of our series of 41 cases.

I Gothenburg, where all autopsies in cluded search for cystic kidneys, 14 cases were encountered among the 3200 utopsies performed t the children hospital and obstetrical departments from 1945 through 1958. This is equivalent t pproximately 1 case per 230 utopsies.

W have divided into four groups the histologically xamined cases in our series. The fourth group contained only four cases of kidneys which in no way resembled each other in appearance. It should be noted that the classification was made exclusively on morphological grounds and consequently should not be made a basis for pathogenetic conclusions. Nor does it make claims to be final. Groups I, II and III have nevertheless a distinct ppearance both grossly and microscopically

Group I

These kidneys usually weigh around 400 g per pair and are considerably over sized but have retained the characteristic shape The surface is smooth and copious masses of tiny cysts are dimly visible through it. On the surface of the section which has a spongy appearance one discerns numerous cystic spaces of round or slot-like shapes situated close together in a radial arrangement extending all the w y from the capsule to the renal pelvis. The renal pelvis is gracile the ureter lender and thin walled (Fig 4).

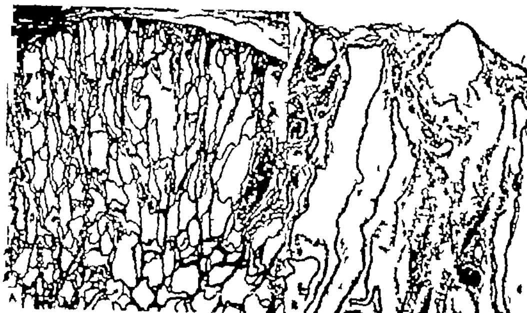


Fig. 5. Polycystic kidney Group I A. 10 B 120.

Microscopical examination discloses in a narrow marginal zone next to the cortex remotely spaced and occasionally incompletely developed glomeruli with not dilated Bowman capsules and a few often poorly developed tubular structures. The rest of the parenchyma is entirely occupied by the aforementioned r und or elongated cystic spaces which are l ned by a regular cubical epith lium In the epithelium th nu lei are rather large the intercellular b rders indistinct (N thing suggest th t the cells are of tubular origin) Down t ward the renal hilu the cyst become m re irregular l tunded larger In juxtacapsular area very mall amount f loose collagen poor tissue is found betw en th s ts but as the renal hilu i pproach d the interstitial tissue becomes more plentiful and richer i collagen fibres Morphologically fully developed nephrons with d fferentiated distal and proximal convoluted tubules are seldom encountered in cystic kidneys belonging to Group I (Fig. 5).

This group is the equivalent of what Sanchez Lucas termed spongy kidneys (rein d éponge).

In three cases belonging to this group histological examination of the liver re vealed a marked proliferation of the finer bile ducts some of which were dilated and pronounced periportal fibrosis (Fig 7) Another four cases were found to have cyst in the liver and one of them in the pancreas too but unfortunately the organs were not examined hi tologically In two cases where pancreas were examined microscopically a marked fibrosis of the parenchyma dilatation of the duct and periductular fibrosis were found (Fig 8)

Cl ical Data

Group I comprised 21 nd girl S x of the hildren were observ Th mselves immediately or shortly after partu it n

Pregnancy and Parturition

Six of the children were born before the 39th week, the remaining three in the 39th–40th week. Four of the children were delivered by breech presentation. From to 4 % of normal births are breech pre sentations (11).

Three parturitions observed by us were associated with hypamnion. In the other cases the amniotic fluid was reportedly normal but nothing was recorded as to its volum Many hypotheses have been presented for the mode of production of amniotic fluid particularly with reference to the part played by the urine (16 31) Several authors have also noted hypamnion in urinary tract malformations and in renal agenesis (7 27) Hypamnion in cases of cystic kidneys has also been observed by Singer (28). The existence of dry labour in the neonatal type of cystic kidneys suggests that such kidneys secrete no urine.

One of the mothers had jaundice during late pregnancy another was an mic and the rest seemed in the best of health.

Symptoms n the Child

The hildren in this group were phyo tic their respiration irregular or hallow and breathing difficulties were d minant throughout the survival period. No child survived more than 4 hours on died immediately after birth none was st ll born.

The most striking feature seen when these children are xamined the greatly distended bdomen nd when t pal pated the kidney re variabl ly f und t be markedl enlarged (F g 3)

Potter described pecif facial



Fig 6. Potters face in case of polycystic kidney Group I.

pression in children with renal agenesis (deep eye furrows, snub-nose micro-gnathia, large flabby ears placed low on the head). But the same thing occurs in other renal malformations too The 6 children we had the opportunity to observe per sonally all had a typical "Potter face" (Fig 6)

Other malformations except for cysts in other parenchymatous organs have been bserved in only one case namely stenosis of the sigmoid flexure

Why these children die so soon after birth is a question few authors have discussed and the general belief seems to be that death occurred a result f uremia. The mere fact that children with renal genesis frequently survive for several days with a slowly rising nonprotein nitrogen levels effectively disproves the view th t renal insufficiency i the cause of death Acid base disequilibrium due to rine retention ould presumably be a ontribut ry cause of death. However



Fig. 7 Duct dilatation and fibrosis in liver (Group I.) 125.

such a disequilibrium would only arise if the placental barrier was damaged. In one of our cases (No 5) a complete blood nalysis was done immediately before ath. The Na, K Co_2 etc levels were te normal for the age and N.P.N was 48 mg %. Dalgaard (6) pointed out that these children presumably die from other causes than their kidney malformation (premature birth pulmonary atelectasis, other malformations incompatible with life)

Cystic kidneys of Group I seem to show something of a hereditary trait Six of our 9 cases occurred in three families (This will be further commented upon).

Group II

Most of these kidneys are considerably enlarged up to 400 g per pair While the typical renal shape usually is preserved, occasional organs may be more or less deformed by large cysts (Fig 9)

Microscopically the cysts exhibit a cuboidal or simple squamous and regular epithelium. The cysts are surrounded by large amounts of fairly loose and moderately collagen rich connective tissue The cysts in some kidneys occupy the greater part of the parenchyma and one find only small residual portions of more normal parenchyma with a few apparently underdeveloped glomeruli and irregular tubular structures next to the cortex In other kidneys those areas which contain cysts and much connective tissues are mixed up with patches of seemingly unchanged renal tissue where there are histologically fully normal nephrons with identifiable proximal and distal convoluted tubules (Fig 10)



Fig. 8. Fibrosis of pancreas. (Group I.)



Fig. 9 Polycystic kidney Group II 0 8.



Fig. 10. Polycystic kidney Group II. A. 10. B 150.

This kidney is indistinguishable from the cystic kidney rich in connective tissue described by Potter It might be identical with the kidney Staemmler described as being morphologically equivalent to the adult type of cystic kidney

Malformations in other organs have not been observed. In the few cases where liver and pancreas were xamined fibrosis of the same kind a in Group I could not be found.

Clinical Data

Included in this group are 10 children (5 boys and 5 girl) from 8 families One of the children (No 7B) is at present 3 years of age with clinical signs (including X ray) of cystic kidneys

Pregnancy and Parturition

Six of the children were born in the 38th week one in the 30th and one in the 39th For the two others nothing is known about the duration of pregnancy

Specific information about the amount of amniotic fluid was available for two cases only it was normal in amount

Six of the children were born in breech presentation, 3 in vertex presentation and in 1 case the mode of presentation was not recorded.

Symptoms in the Child

Apart from Case 7B we personally observed only one of the children in this group Two of the children (Cases 12 and 13) survived the neonatal period and died with renal insufficiency at the ages of 1.5 and 4 months, respectively The remaining six children died within a few hours of birth.

In this group the only other malformation we observed was a case of occipital meningocele and a contributory cause of death was intracranial hemorrhage in Case 11 and hemolytic anemia of newborns in Case 9

Also in children with this type of cystic kidneys the condition appears to have a familial incidence in two families two siblings were affected

Group III

Kidneys in this group are hypopla tic and have cyst of varying siz Occa ionally they become as big as plums and then they deform the organ. Although the parenchyma always is hypoplastic the kidney may have a considerable total weight owing to the presence of very large solitary cysts The uriniferous passages in some cases exhibit severe malformations for example folded or dilated ureters or absence of ureters (Fig 11).

Microscopically the cysts are lined with a squamous or cubical epithelium and surrounded by fairly much connective tissue As a rule one sees only small areas in which there are a few mostly immature glomeruli and in association with these occasional tubular structures (Fig 11) In some cases cartilage was found in the parenchyma.

Included among the hypoplasias by Bell (-), this type of kidney is identical with Staemmler s third type It resembles in many respects the "dysplastic" cystic kidneys in children described by Schwartz (*6) (Cf. also Parkulainen, and Ericsson et al)

Clinical Data

Four boys, two of them uniovular twins, from three families belonged to this group.

Pregnancy and Parturition

One of the children was born in the 39th, the others before the 36th week.

No information regarding the amount of amniotic fluid is available in any of the cases. Micturition definitely occurred in the twins who died after 4 and 7 days. The other children died within one hour

Th pregnancies without exception appeared normal and the children were all born in vertex presentation



Fig. 11 Polycystic kidney Group III. × 6.



Fig 12 Glomerular type of polycystic kidney × 120

Symptoms in the Child

One of the twins (No 16A) had hydronephrosis on the right side in addition to cystic kidneys Moreover urinary tract malformations were observed in the other children of this group

In the first of the twins (No 16A) the existence of renal lesions was not suspected. But as cystic kidneys were found at autopsy an N P.N analysis was made on the second shortly before death the value being 89 mg %. The first twin died after a convulsion the second in conjunction with a cyanotic attack. Both twins suffered from respiratory difficulties and had repeated attacks of cyanosis

Group IV

To this group were assigned four cases that could not be classified under any other group on account of the histological picture In two cases the cysts microscopically consisted of dilated glomeruli, the kidneys accordingly being equivalent to Potter s glomerular type of cystic kidney (Fig 12) In one of these two cases o information was available regarding the ize and gross appearance of the kidneys, in the other they were unequally large and weighed 8 and 260 g respectively In one of the other two cases the kidneys were small and had some cysts in the cortex. Microscopical examination disclosed a cortex and medulla with, on the whole normal histology large numbers of moderately dilated tubular structures being visible along the cortico-medullary border and in the cortex

Clinical Data

This group comprised one boy and three girls. The mothers had apparently normal pregnancies. One of the children with glomerular cysts died a few hours after parturition the other at the age of five years of renal insufficiency The latter child had amaurosis and a cerebral malformation.

Cystic kidney cases not examined histologically by us

Histological examination of the kidneys from 15 children could not be carried out because microscopical slides were not available However all the children concerned had been autopsied at the local hospital, so the diagnosis of cystic kidneys had at any rate been made by gross inspection. In those cases where histological information was available we dared not use it as a basis for classifying the cases into our groups.

Discussion

Morphological remarks

Neonatal and infantile polycystic kidneys exhibit a great variety of gross and microscopic pictures. Yet few authors have attempted to distinguish between morphologically different types. Sanchez-Lucas recognized two distinct varieties of neonatal cystic kidneys, namely one type where the kidney usually is of normal size and has large cysts—similar to our Group III—and another type where the kidney is hyperplastic usually enormously enlarged and has small cysts and a spongy consistence (rein d éponge)—identical with our Group I.

Potter (22) distinguished two different main types one where the amount of connective tissue i normal and another

TABLE 1

No.	Sex	Birth weight g	Weeks of gest.	Presentation Breech = B Vertex = V Caesarian section = C.S.	Survival time	Kidney weight Right Left	Other malformations	No. of sibs	Affected sibs
Group I									
1A	♀	2.190	37	B	3 h	250	Liver cysts	3	1
1B	♀	2.800	36	B	1 h	280	—	3	1
2A	♀	2.690	38	B	1 h	450	Liver cysts, pancreatic cysts	6	1
2B	♀	4.200	35	V	Dead at part.	Very enlarged	Liver cysts	6	1
3	♂	2.900	37	V	4 h	185 210	—	1	0
4	♀	2.800	38	B	1 h	250	—	1	0
5A	♀	3 290	39	V	1 h	460	Fibrosis and duct dilatation of liver and pancreas	3	1
5B	♂	2.900	39	B	1 h	230		3	1
6	♀	2.300	40	V	2 h	105 108	and sigm. stenosis	1	0
Group II									
7A	♀	2.410	35	B	4 h	? ?	—	1	1
7B	♂				Alive			1	1
8A	♂	2.560	34	V	4 h	Large	—	1	1
8B	♀	2.310	39	B	1 h	Large	—	1	1
9	♂	2.700	38	V	5 min.	? ?	Erythroblastosis	1	0
10	♀	2.220	38	B	1 min.	400	—	2	0
11	♂	2.420	37	B	2 h	Large	—	0	0
12	♂	2.770	?	?	3 m	100 100	—	1	0
13	♀	2.350	?	B	4 m	Large	—	1	0
14	♀	2.830	36	V	7 h	80 80	Meningocele + polydactyli	4	0
Group III									
15	♂	2.560	34	V	1 h	0 140	—	4	0
16	♂	3.670	39	V	Dead at part.	Large	—	0	0
17A	♂	2.000	36	V	7 d	Norm. Small	—	2	1
17B	♂	2.100	36	V	4 d	Small	—	2	1
Group IV									
18	♀	2.325	38	B	2 h	6 240	—	0	0
19	♀	4.100	40	?	5 y	? ?	Cerebr. malformation	1	1
20	♀	3.000	38	V	1 h	400	—	2	0
21	♂	2.200	34	V	2 m	Small	—	2	0
Not histologically examined									
22	♀	2.740	36	B	1 h	Large	—	1	0
23	♂	2.250	34	V	1/2 h	? ?	—	1	0
24	♀	4.800	41	C.S.	1 min.	Large	Mongol	0	0
25	♂	2.720	35	B	1 h	? ?	—	0	0
26	♂	1.600	30	B	1 min.	Small	Hydroureter	2	0
27	♀	2.640	35	B	Dead at part.	Very enlarged	—	2	0
28	♂	1.850	37	B	3 h	0 Small	Mongol	3	0
29	♀	1.850	31	V	Dead at part.	650	Encephalocele	0	0

(Table 1 cont.)

No.	Sex	Birth weight g	Weeks of gest.	Presentation Breech = B Vertex = V Caesarian section = C.S.	Survival time	Kidney weight Right Left	Other malformations	No. of sibs	Affected sibs
30	♂	[illegible]600	?	V	1 h	Large	—	1	0
31	♂	2.340	37	?	5 d	Large	Hydronephrosis	1	●
32	♀	3.520	40	V	3 m	Norm. Large	Hydronephrosis	1	●
33	♂	2.050	32	V	d	? ?	Hydronephrosis, Stricture of ureter	0	●
34	♂	3.590	?	?	1 y	Small	Hydronephrosis	2	0
35	♂	3.170	?	?	1 y	Small	—	1	0
36	♂	3.660	?	?	2 y	Large	Hydrocephalus	2	●

where it is greater than normal. In the first category the cysts may consist of either dilated and elongated tubules (our Group I) or—but never *and*—dilated glomeruli (similar to cases in our Group IV) In Potter s second category the cysts are of tubular origin (our Group II)

Using a somewhat different basis for classification, Staemmler (30) distinguished three types of neonatal or infantile cystic kidneys. The first type is characterized by a very large kidney closely resembling that seen in the adult form of the disease (our Group II) the second type exhibits a moderately enlarged kidney of spongy consistence (similar to Sanchez-Lucas rein d éponge and our Group I), the third type is a hypoplastic kidney of irregular configuration (included among the hypoplasias by Bell and our Group III).

The most probable reason why different principles for classification have been employed would seem simply to be that the material available to the various investigators frequently are too small. In most papers dealing with large material the histology has not been fully described.

Cysts in other organs

As many authors have pointed out (for example see Potter) subjects with cystic kidneys frequently have cysts in other organs as well, most often in the liver but also in the lungs and pancreas.

In our material the findings of cysts in other organs are strictly limited to cases belonging to Group I In this group we have by microscopical examination observed a proliferation of bile ducts and periportal cirrhosis in all examined cases who did not exhibit gross cysts in the liver These findings are in agreement with Potter s. Moreover we have observed fibrosis and duct dilatation in pancreas in two cases belonging to Group I.

Pathogenesis

Numerous papers have been devoted to the formal genesis of cystic kidneys. The problem has been attacked along two main lines. Either the type of the cyst epithelium as it appears under the microscope has been used to trace the origin of the cysts to certain structures, or microdissection and serial sectioning with subsequent reconstruction have been employed in at

tempts to derive the cysts from the various components of the nephron.

Using the reconstruction method in two cases of neonatal cystic kidneys, Lambert (16) discovered that the cysts were of glomerular tubular and possibly also collecting tubule origin. He stated that the "cystic nephrons invariably were separated from their secretory tubules"

Bialestock (3) applied the microdissection technique in one case of cystic kidneys from a prematurely born child, finding that most of the cysts were derived from the glomerules and some most likely from the tubules. Also the non-cystic nephrons exhibited changes in the form of short irregular and thin walled tubules. Bialestock was unable to confirm Lambert's observation that the cystic nephrons lacked communication with the collecting tubules.

As it is impossible to tell from the morphological observations reported by these two authors what type of cystic kidney they investigated, their cases can not be fitted into the classification adopted by us. Most likely they were observing quite different types of cystic kidneys. Nevertheless, although they warrant no generalized conclusions regarding the formal genesis of cystic kidneys, their findings do indicate that the cysts may originate from different parts of the nephron.

Different types of cysts can seldom be recognized solely on the basis of histological appearances. The large majority of cysts are lined with either cuboidal or pavement epithelium lacking characteristic morphological features. The appearance of such epithelium obviously provides no clues as to whether the cyst originates from a particular part of the nephron. The histological appearance is specific only in those cases where one definitely sees glomerular cysts with glomerular coils in the wall and also in those cases where the cysts are tubular and occur together with cystically dilated tubules and fully normal tubular structures.

Such morphological observations combined with known facts about the normal evolution of the kidney have been the basis for many published theories regarding the genesis of cystic kidneys. As early as 1894 Hildebrand (1*) presented the theory which was later supported by Ribbert (24) that various nephronic components arising from the metanephrogenous blastema receive no communication to the collecting tubules so that urine secreted from the glomeruli is not drained away and accumulates in the nephron which ultimately leads to the formation of a cyst.

Kampmeier (13) expressed the opinion that the first generations of glomeruli formed in the kidney normally degenerate a process which often can give origin to small residual cystic structures. The causative factor of polycystic kidney might thus be exaggeration of the normally occurring, mild cystic degeneration of embryonic glomeruli (14).

Other authors have drawn attention to the possibility that interstitial inflammation might cause occlusion of the tubules (1).

Cystic kidneys have also been interpreted as a neoplastic abnormality secondary to pathological epithelial proliferation in the tubules (5, 20).

As pointed out by a few authors who have classified various types of cystic kidneys, there is no reason to believe that all types necessarily must have either the

same etiology or the same formal genesis (22, 30) Potter studied 50 cystic kidneys from newborns and infants without finding anything in support of either of the two most common theories regarding the etiology of the abnormality She pointed out that abnormal development of various parts of the nephron may produce aberrations of a type that would enable cysts to be formed. As other organs too often exhibit changes with formation of cysts, the process seems to be one that is not confined exclusively to the kidneys. This applies particularly to cystic kidneys of Group I in our classification.

Etiology

Occasional cases have been described in which the mother of a child with cystic kidneys had german measles during pregnancy (19) In our series only two of 36 mothers had been exposed to german measles during pregnancy (in the 2nd and 4th month respectively) without clinical signs infection One mother had erythema nodosum in the 2nd month, the others were all healthy during the first half of pregnancy It can safely be assumed that any viral disease occurring during pregnancy would have been noted, at least in the mothers of children born later than 1950 because after that year viral diseases in pregnant women have attracted particular interest in this country

Although many authors have postulated that cystic kidneys in infants are inherited recessively (6 20 33) no study seems to have been carried out on a morphologically uniform material. The present phase of our researche concerning cystic kidneys includes superficial genealogical analysis. The preliminary investigation—consisting of simple interviews with the parents—disclosed no evidence of cystic kidneys occurring in ancestors or relatives, nor did it provide proof of any consanguinous marriages.

However when in the Group I type malformation, where the children are unviable and die within a few hours after birth, there is a familial occurrence, a recessive heredity must be assumed. Our Group I includes 9 cases of polycystic kidneys among 21 sibs. When the figures are treated for the selection bias in principle according to Weinberg (32) the corrected figures will be 6 affected among 27 sibs (Table 2)

Thus, the affected comprise 22.2% of all sibs, corresponding to the expected 25% is as good as can reasonably be demanded. It has consequently been shown that the manifestation frequency in our Group I agrees with the assumption of the heredity of a single recessive gene. The risk for a family with this recessive gene of having an abnormal child is accordingly one in four The prognosis for the affected child is grave. For siblings who do not exhibit the clinical symptoms described in the foregoing and do survive the first day or days the risk of their having cystic kidneys is nil but, in accordance with the above assumptions, two thirds of the healthy children will carry the gene.

In practice this implies that when a case of cystic kidneys of Group I has occurred the prognosis for subsequent children is reasonably favourable and so the parents, provided they are aware of and understand the risks involved need not be cautioned against having any more children. Notably however the healthy chil-

Table 2. *Group I Correction for the selection bias according to Weinberg*

Conductors	N of children	Affected (A)	Unaffected (U)	A (A 1)	AU
1 A, B	4	2	2	2	4
2 A, B	7		5	2	10
3		1	1	0	1
4	2	1	1	0	1
5 A, B	4	2	2	2	4
6	2	1	1	0	1
Total	21	9	12	6	21

dren should be strongly discouraged from contracting consanguineous marriages.

Group II includes 10 cases among 19 sibs. After correction as above the affected comprise 18.1 %. As, in this group the kidneys have a more or less intact parenchyma enabling the subjects in some cases to live for weeks or years, and we have investigated only few sibs we cannot use this figure as a proof of recessive heredity Thus the heredity is not fully understood, but the familial occurrence is documented and the affected child can sometimes live for quite a long time This must be considered in discussing additional children with the parents.

Summary

Twenty-eight cases of polycystic kidneys in newborn, infants and children have been investigated clinically and histopathologically Three more or less distinct morphological types of polycystic kidneys are described and in addition single cases of deviating appearance The clinical and hereditary aspects are discussed. In Group I (spongy kidney) a single gene recessive heredity has been proved.

References

1 Arnold J Über angeborene einseitige Nierenschrumpfung mit Cystenbildung Beitr Path Anat, 8 21 1890.
2. Bell, E. T Renal Diseases. Philadelphia 1947
3. Bialestock, D The morphogenesis of renal cysts in the stillborn studied by microdissection technique. J Path Bact, 71 51, 1956.
4. Bricker, N S. and Patton J F Cystic disease of the kidneys. Amer J Med, 18 207 1955.
5. Bambie, V and Severi, A.. Contributo alla patogenesi delle cisti renali. Sperimentale, 40 1 1890.
6. Dalgaard, O Z.: Bilateral Polycystic Disease of the Kidney Diss. Munksgaard, Copenhagen 1957
7. D vis, E. and Potter, E.: Congenital malformations and obstetrics. Pediatrics, 19 719, 1957
8. Ericsson N O. and Ivemark, B. I.: Renal dysplasia and pyelonephritis in infants and children. Arch Path, 66 264, 1958.
9. Fergusson J D Observations on familial polycystic disease of the kidney Proc Roy Soc Med, 42 806, 1949.
10. — Observations on familial polycystic disease of the kidney Brit J Urol, 21 241 1949
11. Hedberg, E.: Breech deliveries. Acta Obstet Gynec Scand, 16 356, 1937
12. Hildebrand: Weiterer Beitrag zur pathol. Anatomie der Nierengeschwülst Arch kl Chir 48. 343, 1894.
13. Kampmeier, O. F A hitherto unrecognized mode of origin of congenital cysts. Surg Gynec Obstet, 36 208, 1923.

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1 A, B	4			2	4
2 A, B	7		5	2	10
3	2	1	1	0	1
4		1	1	0	1
5 A, B	4	2	2		4
6		1	1	0	1
Total	21	9	12	6	1

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References

1. ARNOLD, J Über angeborene einseitige Nierenschrumpfung mit Cystenbildung *Beitr Path Anat, 8* 1, 1890.
2. BELL, E. T Renal Diseases. Philadelphia 1947
3. BIALESTOCK, D.: The morphogenesis of renal cysts in the stillborn studied by microdissection technique. *J Path Bact, 71* 51 1956.
4. BRICKER, N S. and PATTON J F Cystic disease of the kidneys. *Amer J Med, 18* 207 1955.
5. BRUSINI, V and SEVERI, A Contributo alla patogenesi delle cisti renali. *Sperimentale 46* 1 1890.
6. DALGAARD, O. Z.: Bilateral Polycystic Disease of the Kidney Diss. Munksgaard, Copenhagen 1957
7. DAVIS, E. and POTTER, E.: Congenital malformations and obstetrics. *Pediatrics, 19* 719 1957
8. ERICSSON, N O and IVEMARK, B. I.: Renal dysplasia and pyelonephritis in infants and children. *Arch Path, 66* 264 1958.
9. FERGUSSON J D Observations on familial polycystic disease of the kidney *Proc Roy Soc Med, 42* 806, 1949.
10. — Observations on familial polycystic disease of the kidney *Brit J Urol, 21* 41 1949
11. HEDBERG, E.: Breech deliveries. *Acta Obstet Gynec Scand, 36* 336, 1957
12. HILDEBRAND: Weiterer Beitrag zur pathol. Anatomie der Nierengeschwülste *Arch Klin Chir 48* 343, 1894
13. KAMPMEIER, O F A hitherto unrecognized mode of origin of congenital cysts. *Surg Gynec Obstet, 36* 208, 1923.

14. Kampmeier, O F and McKenna, C. M.: A consideration of the development of polycystic kidney *J Urol, 32* 37 1934.
15. Kinnunen O.: Fostervattnets uppkomst *Nord Med, 46* 56, 1951
16. Kinnunen O and Paalanen A.: Fostrets njurfunktion. *Nord Med, 47* 19 1952
17. Küster, E.: Die chirurgischen Krankheiten der Niere *Deutsch Chi 52 B* 512, 1896-1902.
18. Lambert P Polycystic disease of kidney *Arch Path, 44* 34 1947
19. Lipton E. L. and Scordamaglia, L. J.: Congenital unilateral multicystic kidney associated with maternal rubella. *J Pediat 50* 730 1957
20. Olow I.: Congenitala cystnjurar *Nord Med, 54* 1461 1955.
21. Parkkulainen K. V., Hjelt L. and Sirola K.: Congenital multicystic dysplasia of the kidney *Acta Chi Scand, Suppl 244* 1959.
22. Potter, E.: Pathology of the Fetus and the Newborn. Chicago 1952.
23. Rall, J E. and Odel, H. M. Congenital polycystic disease of the kidney: review of the literature and data on 207 cases. *Amer J Med Sc, 218:* 399 1949
24. Ribbert H.: Über die Entstehung der Cystenniere. *Verhandl Deutsch Path Gesellsch,* 187 1900.
25. Sanchez-Lucas, J G.: Morphogenèse de rein polykystique *Sém Hop Paris, 27* 793, 1951
26. Schwartz, J Unusual unilateral multicystic kidney in infants. *J Urol, 35* 259, 1936.
27. Seeley G W and Parmelee, A.: Bilateral renal agenesis and oligohydramnios. *J Pediat 48* 70, 1956.
28. Singer, H. A. Kongenitale Cystenniere beim Neugeborenen *Surg Gynec Obst. 38* 768 1924
29. Sorsby L: Clinical Genetics. Butterworth London 1953.
30. Stammler, M. in Kaufmann, E and Staemmler, M.: Lehrbuch der speziellen pathologischen Anatomie Berlin 1957.
31. Thierstein S., Coleman F and Turner, F.: Kidney function in the fetus. *Amer J Obstet 34* 1178, 1948.
32. Weinberg W Auslesewirkungen bei biologisch-statistischen Problemen. *Arch Rass u. Ges Biol,* 417 1913.
33. Wenger, M.: Erbbiologie und Erbpathologie des Harnapparates. Handb. der Erbbiol. des Menschen. Berlin 1944

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SUMMARY OF SUPPLEMENT

Studies of Hypothyroidism in Children

by HENNING ANDERSEN

(Supplement 125)

In the *introduction*, *hypothyroidism* in children is defined as any hypofunction of the thyroid gland which affects the growth and development of the child. The designation is used to describe the clinical picture throughout the whole course of childhood, instead of such names as congenital and acquired myx oedema, cretinism, etc. *Athyrosis* is the most severe degree of hypothyroidism.

The work consisted in the main of examining a series of hypothyroid children by means of tests with radioactive iodine supplemented by other studies, in the first instance examinations of the bones and teeth. The aim was, first to arrive at as early and sure a diagnosis as possible, and secondly to try and sort the hypothyroid children into as uniform groups as possible according to type and degree of thyroid defect with the intention of arriving at an improved evaluation of the progress, the effect of treatment, etc.

Chapter I gives the history of hypothyroidism and the various views on it etiology. The literature on the mental prognosis for these children is also reviewed here, this being the most important and most discussed clinical problem.

Chapter II reviews studies of the uptake of radioactive iodine (I^{131}) in euthyroid children. The author's own procedure is described. (Minimal, 0.25–2 μc., doses of I^{131} have been used, far less than in most other studies.) In 44 euthyroid patients aged 8 months to 17 years, whose symptoms had suggested hypothyroidism, the mean percentage uptake of I^{131} was found to be 43.5 ± 1.5 after 24 hours, with a standard deviation of 9.7.

Chapter III discusses previous studies including those by the author on the uptake of I^{131} in hypothyroid patients. The author's material comprises all those children with primary (thyrogenic) hypothyroidism, examined by mean of the above-mentioned technique at Queen Louise's Children's Hospital during the last five years. Cases of secondary (hypopituitary) hypothyroidism are not included in the present material. Three groups could be distinguished.

Group I. 24 children with very reduced or no uptake of I^{131} over the thyroid gland.

Group II. 14 children with exclusively ectopic sublingual I^{131} uptake.

Group III. 18 children with hypothyroidism and goitre.

This distribution deviates considerably

14 KAMPMEIER, O F and MCKENNA, C. M.: A consideration of the development of polycystic kidney *J Urol*, *32* 37 1934

15 KINNUNEN O.: Fosterv tinets uppkomst. *Nord Med*, *45* 56, 1951.

16 KINNUNEN O and PAALANEN A.: Fostrets njurfunktion *Nord Med*, *47* 19 1952.

17 KÜSTER, E.: Die chirurgischen Krankheiten der Niere *Deutsch Chir* *52 B* 51.., 1896–1902.

18 LAMBERT P.: Polycystic disease of kidney *Arch Path*, *44* 34 1947

19 LIPTON E. L. and SCORAN VAGLIA, L. J.: Congenital unilateral multicystic kidney associated with maternal rubella. *J Pediat* *50* 730 1957

20. OLOW I.: Congenitala cystnjurar *Nord Med* *54* 1461 1955.

21 PARKKULAINEN K. V HJELT L. and SIROLA, K.: Congenital multicystic dysplasia of the kidney *Acta Chir Scand* Suppl *244* 1959

22. POTTER, E.: Pathology of the Fetus and the Newborn. Chicago 195..

23. RALL, J E. and ODEL, H. M.: Congenital polycystic disease of the kidney: review of the literature and data on 207 cases. *Amer J Med Sc* *218* 399 1949.

24. RIBBERT H.: Über die Entstehung der Cystenniere. *Verhandl Deutsch Path Gesellsch*, 2 187 1900.

25. SANCHEZ LUCAS, J G Morphogenèse de rein polykystique. *Sem Hop Paris*, *27* 2793, 1951

26. SCHWARTZ, J Unusual unilateral multicystic kidney in infants. *J Urol*, *35* ?78, 1936.

27 SELBY G W and PARMELEE, A.: Bilateral renal agenesis and oligohydramnios. *J Pediat* *48* 70, 1956.

28. SIEGEL, H A.: Kongenitale Cystenniere beim Neugeborenen. *Surg Gynec Obst* *38*. 768, 1924

29 SORSBY I Clinical Genetics. Butterworth London 1953.

30. STAEMMLER, M. in KAUFMANN E. and STAEMMLER, M.: Lehrbuch der speziellen pathologischen Anatomie, Berlin 1957.

31 THIERSTEIN S. COLEMAN F and TAPLEY, F.: Kidney function in the fetus *Amer J Obstet*, *56* 1178 1948

32. WEINBERG W Auslesewirkungen bei biologisch-statistischen Problemen. *Arch Rass & Ges Biol*, 417 1913.

33. WERNER, M.: Erbbiologie und Erbpathologie des Harnapparates. Handb. der Erbbiol. des Menschen, Berlin 1944

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together is that usually given in the literature: twice as many girls as boys. However if the cases diagnosed before the age of 6 months are considered the distribution is found to be *equal* between the sexes, while for the cases diagnosed after the age of 6 months, the proportion is still about twice as many girls as boys. Grouping a number of similar series together the same difference is apparent and appears to be significant at the 5 per cent level.

Chapter V commences with a description of the *normal hormone production of the thyroid gland* and the disturbances in the production, either as a result of lack of iodine (*endemic cretinism*), or *disturbances of hormone synthesis* on account of congenital enzyme defect and the like.

Group III The author describes *18 patients with hypothyroidism and goitre*, and attention is drawn for one thing to the pronounced *familial disposition* and to the occurrence of a *large number of deaf mute children* (about ⅓) in this group. The reason for this is discussed. At the time of diagnosis the children in Group III are on the average older than the children in Group I. The age distribution is illustrated for all three groups.

The various uptake curves for I^{131} are discussed, and the histological findings described in some thyroidectomized patients in this group.

Chapter VI on the basis of a review of the literature discusses hypothyroidism as a sequela of extrathyroidal enzyme defect, and hypothyroidism as a sequela of auto-immunization (Hashimoto's struma).

Chapter VII discusses the relationship between "simple" goitre and hypothyroidism with goitre. The author studies of 28 euthyroid children with goitre are discussed, with an account of familial incidence, uptake of I^{131}, content of protein-bound iodine in serum, etc.

Chapter VIII discusses the X ray changes in hypothyroid children. The typical epiphysial changes in the long bones, the "dysgeneses" are mentioned, together with the diagnostic significance of these findings. Further cases are described of *a type of euthyroid child of hypothyroid habitus with "peripheral dysostosis" of the toes, a condition apparently not previously mentioned in the literature.* These children had all been erroneously regarded as hypothyroid.

The X ray changes in the spine are described, both the localised deformities at the transition between the lumbar and thoracic spine and—more characteristic—the general retardation of the spine. These changes progress more or less parallel with the retardation in the peripheral bone development. A spinal type of "dysgenesis" of the vertebral body was described by the author in 1954. These findings are discussed.

The roentgenological picture of the cranium in hypothyroidism is described, with particular reference to the size of the sella turcica, and the significance of this is discussed.

An unusual case is presented of apparent temporary osteopetrosis in an athyreotic infant who at the same time showed persisting changes in the enamel of the teeth.

Chapter IX reviews the author's studies of the teeth in hypothyroid children. It is stressed that the size, number and shape of the teeth are normal, whereas the enamel is hypoplastic and dysgenetic. This

fits in with the times for the appearance of the tooth "anlage" enamel formation and foetal thyroid gland function. *Studies of the frequency of enamel hypoplasias (present in about 80 per cent of the hypothyroid children examined) may be used for example, to estimate whether hypothyroidism has been present prenatally as such changes (acquired in utero)* are very rarely observed in normal children, or in children with other diseases, and the changes do not disappear during thyroid treatment.

Dental development is, in general, less sensitive to changes in the thyroid status of the organism than bone development. The same is also the case for other hormones for example sex hormones and adrenocortical hormones.

Chapter X describes previous studies of foetal I^{131} uptake in man. The conclusion of the author's studies is that the foetus takes up I^{131} in the thyroid gland—but not in demonstrable amounts at other sites—from about the 12th–14th week of pregnancy and that the gland appears to be very active.

The *final remarks* discuss in particular the question of early diagnosis, together with the possibilities of genetic prophylaxis and intrauterine treatment of the foetus by treating the mother.

Acta Paediatrica 50: 205–215 March 1961

PROCEEDINGS OF PEDIATRIC SOCIETIES

The Pediatric Society of South Sweden

Meeting, May 8, 1960

R. Zetterström: Hypoglycemia in Children. (Will be published elsewhere.)

Per Kählin: Neurologic Symptoms Am ng Juvenile Diabetics.

Of 100 diabetic children born since 1943 examined at Gothenburg Children's Hospi tal 8 had on one or more occasions grand mal seizures. These had occurred in connection with hypoglycemia as well as without any certain hypoglycemic symptoms. At the time of the seizures in all 8 children their insulin supply was poorly regulated. They frequently had pronounced glycosuria at the same time as insulin reactions. The disposi tion to seizures decreased or disappeared when the insulin dose, in spite of the glycosuria, was reduced. Thus, the possibility of overinsulinization as a cause of the seizures appears good. The insulin-induced hypoglycemia can cause discharge of adrenalin, which in turn leads to increased mobilization of glucose (Somogyi: *Amer J Med* 26 1 1959). It is possible that repeated hypoglycemic attacks produce cerebral lesions which increase the patient disposition to seizures. Changing the type of insulin did not have any definit influence on the seizures. Peroral preparations have not been employed. EEG examinations, occurrence of convulsions prior to the onset of diabetes and the heredity of the patient yielded no certain evidence for the diagnosis of epilepsy. However the EEG examinations revealed a high frequency of unspecific abnormality and in two cases paroxysmal activity among the 8 children with convulsions and the 26 other children with labile diabetes. This has been previously observed in older diabetics and their relatives (Izzo- *Diabetes*, 2: 93, 1953, Greenblatt. *New Engl J Med*, 234 119, 1946; Andersson–Kirstein. *Dtsch Med Wschr*, I 13, 1959). N focal EEG changes were noticed. The EEG changes may be mani festation of a posthypoglycemic encephalopathy may be due to genetic factors or in older patients with focal changes may be a sign of changes in the cerebral blood vessels.

G Berglund and *B Lindquist* Generalized Diseases of Bones in Children. (T be pub lished in Nord Med.)

S. P Fällström and *T Reinand* Gastric and Duodenal Ulcer in Children. Clinical and Psychiatric aspects.

A series of 36 children with proven peptic ulcers has been reviewed, and a number of the most recent cases were made the object of special psychiatric-psychologic examinations. Thirty five children were of school age There is a marked predominance for boys. The frequency of ulcers seems t have increased in older ch ldren. These children often have a strikingly typical ulcer anamnesis. Complications have consisted of hemor rhage in 5 cases and stenosis in 2 cases. The immediat therapeutic results have been satisfactory in most cases. The frequency f recurrence, however is high. Constitutional and hereditary factors are significant for the occurrence of ulcers in children. Various characteristics connected with the dispos tion to ulcers are common among these cases. The environmental factors are not equally conspicuous. Neurotic characteristics or emotional disturbances could not be demonstrated t any greater extent in these children.

Per Selander Malmö

Swedish Pediatric Society

Meeting March 11 1960

A. Alvin and R. Tunell Sternal elevation — an attempt to treat pulmonary atelectasis in newborns

In newborn infants and particularly prematures the fragile and soft thoracic cage may result in pronounced retractions of the lower third of the sternal region, especially in those infants with pulmonary atelectasis. This condition has been compared to that seen in patients with the crushed chest syndrome in whom sternal elevation has proved beneficial. W have therefore treated five newborns with sternal levation. All had generalized cyanosis, pronounced retractions of the lower third of the sternal region and roentgenological evidence of pulmonary infiltrations.

Technique. A double peafil-string suture was placed through the periosteum of the sternum immediat ly above the ensiform process. The suture was attached to the top of the incubator by a rubber board and the tension so adjusted that the thoracic cage assumed a normal shape. The sternum was kept in this position for 48 hours; in two treatment was reinstituted a few later because of recurrence. In three *of the five infants the immediate results* were good. These infants had birthweights of 3500, 2170 and 2060 g In two there was a fall in the respiratory rat and in all three there was a clear clinical improvement after the application f the thoracic elevation. In the other two cases the treatment was with out any clinical effect and the children died within 2 days after birth. Post mortem ex amination revealed hyaline membranes in both and one had an intracranial haemor rhage as well. It is our intention to continue using *sternal elevation in newborn children* with the triad of general cyanosis, marked retractions of the lower third of the sternal region and roentgenological signs of pul monary atelectasis. The management may preferably be restricted to children with a birthweight of more than 2000 g.

A. J W Hagströmer P ternal age and "fetopathy"

Advanced paternal age has been shown to be a feature in the etiology of achondroplasia (L. S. Penrose 1957 and W Lenz, 1958). A prospective investigation to evaluate the relation between paternal age and "fetopathy in general" has therefore been carried out. The term "fetopathy" as used here includes malformation, stillbirth, neonatal death and/or intracranial hemorrhage. The occurrence of fetopathy at advanced pater nal age (i.e. the supposed father over 50 years of age) was compared to that found at low paternal age (i.e. the supposed father under 50 years of age) in a control material. In both groups the mothers were of the same age, parity and social status. The offspring of unmarried mothers were excluded from the study The incidence of fetopathy in the group of 341 fathers over 50 years of age was found to be 6%. In the offspring of 752 mothers married to men less than 50 years of age the incidence of fetopathy was also found to be 6%. *The influence of paternal age on the incidence of fetopathy registered at birth is thus too slight to be detected in the present quantity f data.*

A. Alvin. Successful treatment of listeriosis in newborns

Two cases of listeriosis in newborns are presented. The symptoms were those usual for this age, i.e acute meningitis and sepsis.

Case 1: A girl, born 6 weeks before term weighed 2420 g at birth. During pregnancy the mother had several episodes of bleeding at the time of expected menstruation. From the tenth to the third day prior to delivery she had a septic fever with conjunctivitis, but had no signs of upper respiratory infection, or headache No treatment was given. On the second day of lif the infant became cyanotic and wa *admitted* t the Sachs Children Hospital Multiple pinpoint erythematopapula *lesions* were pre-

sent over the entire body Treatment with tetracycline was instituted at once and con tinued for eleven days. Lumbar puncture revealed 580 white cells per mm³ of which 540 were mononuclears. The recovery was uneventful. Three weeks post partum serological examination of the mother revealed an agglutination titer of 1/64 against *Listeria monocytogenes* and a CF titer of 1/160. One month later these values had fallen to 1/3_ and 1/15, respectively The infant was subsequently examined at six months and appeared well.

Case 2 A full-term male infant, the product of normal delivery was admitted on the second day of life because of cyanosis. Multiple maculopapular lesions were present over the body Examination of the cerebrospinal fluid revealed 2400 whit cells per mm³ and *Listeria monocytogenes* was isolated on culture both from spinal fluid and from the pharynx. Treatment with chloramphenicol (1 days) and a sulfa preparation (3 eeks) was instituted. The recovery was complet and on examination at six months of age the infant appeared well. The mother from the fifteenth t the sixth day prior to delivery had a slight common cold. Four eeks after delivery the mother serum showed an agglutination titer against *Listeria* of 1 _56 and CF titer of 1 240

Listeriosis is considered responsible for about 1 of neonatal deaths. Early diagnosis and treat nent ppears essential to reduce the mortality figure as well as to prevent possible nursery outbreak Secondary infection of other infan and of nursing personnel in the nursery ha been observed by others Diagnosis of listeriosis during pregnancy is discussed. Treatment instituted at this time seems to protect the child against infection.

Discussion: *A. Wallgren* Can *Listeria* infection in mothers be transmitted to their foetuses during subsequent pregnancies? Or is the risk only present when the infec tion is newly acquired as in toxoplasmosis? Could repeated bortions as in this case be caused by chronic listeriosis in the mother? — *A. Alvin*. Yes, there are case reports suggesting this. The theory is that the agents colonize in the vaginal mucous membrane Seeliger has reported 8 patients with habitual abortions in whom serological evidence of Listeria infection was found. After antibiotic treatment 6 of the women gave birth t normal, healthy children.

I Alm G Rodh and F Edvall Current social and medical problems in mothers homes

Discussion: *A Wallgren* It is regrettable that all training schools for unmarried mothers in Stockholm are now transformed in mothers homes. These training schools gave these young unmarried and inexperienced mothers a proper training for the care of their child and in house-keeping. In the past there was much discussion if mothers should be forced t stay in these institutions during the whole breastfeeding period or if they should have free hoice of bo long they want t stay in the institutions.

The community now unfortunat ly has taken way great part of the mothers responsibilit as regards breastfeeding and the general care of the child I think we pediatricians gree that this trend is not beneficial for the hild.

Meeting April 8, 1960

B Ivemark and L. Sirén Cachexia resulting from infundibuloma

The patien was born t term and devel oped normally unt l the age of four months. She hen became anorexic and lost weight At he age of seven months she was admitted t the Samariten Children Hospital in Stockholm. On examination she weighed 4500 g, was poorly developed but was other wise normal. Routine laboratory xaminations of blood, urine and feces were also normal. The electroencephalogram was suggest of right-sided brain lesion. She

remained in the hospital for 15 months and during this time gained only 7000 g. She died at the age of 22 months without any definite diagnosis having been made. On post-mortem examination a grey lobulated, sharply demarcated, non-capsulated tumor the size of an orange was found in the region of the thalamus and the hypothalamus. On section, the surface was homogeneous, moist and some areas were gelatinous and vascular. On microscopic examination the tumor was highly vascular with uni- and bipolar cells containing fusi form nuclei. In the gelatinous areas mucoid degeneration and large round cells with eosinophilic granules resembling histiocytes were found. Some eosinophilic round formations without nuclei were also detected but no deposition of mineral salts had occurred. The structure of this tumor corresponds to that described as an infundibuloma by Globus (*J Neuropath Exp Neurol* 1 59 1942). The clinical picture is generally that of a diencephalic syndrome with onset in early infancy. The course is often protracted and characterized by euphoria and cachexia. Endocrinological symptoms are rare.

I. Gallmar and A. Rack-Nerlund. A case of meningococcal meningitis with uncommon complications.

A ten year-old, normally developed boy who was known to have a systolic murmur possibly due to ventricular septal defect became ill with symptoms of meningococcal meningitis and was admitted after two days. At admission he had cutaneous bleeding but his general condition was rather good and there were no signs of shock. Treatment was immediately started with large doses of penicillin and sulfa and after three days the temperature was normal and the skin lesions healed. About 36 hours later fever recurred associated with signs of pleurisy pericarditis, myocarditis and possible endocarditis, and arthralgia and swelling of the large joints including the elbow. The antistreptolysin titre was 1100 IU/ml; however no hemolytic streptococci were cultured from the throat. In about two weeks the patient improved, was afebrile and the ESR was only slightly elevated. The antimicrobial dosages were reduced but 10 days later the patient again developed high fever, arthralgia and signs of carditis. The doses of penicillin and sulfa were increased without effect, following which cortisone was added and the symptoms quickly subsided. The differential diagnostic possibilities include: 1. septic metastases, 2. toxemia, 3. drug allergy; 4. toxic allergic reaction resembling rheumatic fever.

DISCUSSION: *E. Bengtsson.* A case of meningococcal meningitis associated with arthritis and myocarditis was seen this fall at the Hospital for Infectious Diseases. Signs of residual cardiac involvement are still present. In 1953 during the epidemic of paratyphus fever among 800 consecutive cases of paratyphus fever type Breslau, 10 patients had an associated polyarthritis with high sedimentation rate. None presented an elevated ASLO titer and none had any evidence of cardiac involvement either during the acute episode or at follow-up 4 to 5 years later. There was never any reason to suspect rheumatic fever. It is extremely rare that any organism other than streptococcus is associated with rheumatic fever. The case presented here may be an exception. On the other hand, such marked elevation of the ASLO titer even if not significantly increased makes it difficult to rule out a streptococcal infection.

Meeting May 7 1960

O. Celander. Studies of the peripheral circulation in newborns. Published in Acta Paediat, 49: 488, 1960.

O. Celander and G. Thunell. Method for measuring the blood pressure in newborn infant. Published in Acta Paediat, 19: 497, 1960.

J Bjure, G. Lidén, T Reinand and A. Lesby Follow-up study of icteric fullterm infants without hemolytic disorders. Will be published in Acta Pædiat.

L. Nilsson Fanconi's anemia and various types of this syndrome. Published in Acta Pædiat, 49: 518, 1960.

H A. Hansen, R. Jagenburg B Johansson and T Reinand Hemoglobinopathia M Published in Acta Pædi t, 49: 503 1960.

H Forssman and O Lehmann Chromosom studies in mongolism. Published in A t Pædiat, 49: 536 1960

Meeting Sept. 26 1960

Hypoglycemia childhood (Symposium at the Children Hospital, Gothenburg) Members of the panel. O Broberger O Lehmann nd B Redm. Moderator: Prof. R. Zetterström. Will be published in Acta Pædiat, 1961

Meeting Sept 28 1960

Gerta C. Arneil (Glasgow): Traumatic osteodysplasia

Ten infants (3 males, 7 females) whose ages ranged from 1–24 months, are described in whom degree of trauma varying from unrecognised to considerable led to a typical pattern. The condition was variously misdiagnosed as scurvy neonatal osteitis, hemophilia, cortical hyperostoses, leukemic osteitis and congenital syphilis by senior experienced clinicians. The pattern of disorder is minor fracture involving the diaphyseal portion of the metaphysis Extensive subperiosteal hemorrhage occurs and strips the loosed attached periosteum. Squaring and calcifying of the subperiosteal hemorrhage leads to gross deformity of bones which may take years t heal. Multiple deformities are common and may be seriatum rather than concurrent. By a process of evolusion it has been concluded that this pattern may be due either to undue trauma on a normal skeletal system or slight trauma where osseous stability is impaired. The stages of the disease an abnormal response of normal osseous tissue are: (1) traumatic damage to the diaphyseal plate, (2) considerable subperiosteal hemorrhage, (3) calcification of hemorrhage and squaring of ends of bone, (4) bizarre pattern of metaphyseal structure and (5) recovery

W P Sweetnam (Huddersfield): Congenital fibrosis of the liver as familial d fect

Congenital cystic disease of the liver is ell known. A variant of the condition, congenital fibrosis of the liver is becoming increasingly recognised. In congenital f brosis of the liver there is an increase of fibrosis in the portal tracts together with an excess of small bile ducts, the zonal pattern being otherwise normal. It seems likely that the progressive dilatation of the bile ducts and the attenuation of the fibrous tissue finally gives rise t the typical polycystic li er There may be an associated polycystic disease of the kidneys (Melnick 1955, Parker 1955) and of the pancreas (Ivemark *et al* 1959). The syndrome may present t birth or may be an incidental finding at autopsy Sherlock (1960) has records of two families in whom all the siblings are affected. This report describes the condition in all three children of one family Biopsy in each case showed a typical picture of congenital fibrosis of the liver The first two cases presented t the ages of two and five with hematemesis. The presence of portal hypertension, esophageal varices and portal-systemic collateral circulations were demonstrated by percutaneous transplenic portal venography Both children were protected from further hemorrhage by porta-caval side-to-side anastamoses and have remained well for 6½ and 7 years. One of these children has polycystic kidneys The third child died at the age of six months from pyelonephritis due t polycystic kid neys. It is suggested that congenital fibrosis of the liver is a genetically determined de fect and may be dominant

Diabetes in children and adolescents (Symposium at the Crown Princess Lovisa Chil

remained in the hospital for 15 months and during this time gained only 2000 g. She died at the age of 22 months without any definite diagnosis having been made. On post mortem examination a grey lobulated, sharply demarcated, non-capsulated tumor the size of an orange was found in the region of the thalamus and the hypothalamus. On section, the surface was homogeneous, moist and some areas were gelatinous and vascular. On microscopic examination the tumor was highly vascular with uni- and bipolar cells containing fusiform nuclei. In the gelatinous areas mucoid degeneration and large round cells with eosinophilic granules resembling histiocytes were found. Some eosinophilic round formations without nuclei were also detected but no deposition of mineral salts had occurred. The structure of this tumor corresponds to that described as an infundibuloma by Globus (*J Neuropath Exp Neurol* 1 59, 1942). The clinical picture is generally that of a diencephalic syndrome with onset in early infancy. The course is often protracted and characterized by euphoria and cachexia. Endocrinological symptoms are rare.

Gullmo and A Roch Norlund. A case ...coccal meningitis with uncommon ...cations

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Ten infants (3 males, 7 females), whose ages ranged from 1–24 months, are described in whom a degree of trauma varying from unrecognised to considerable led to a typical pattern. The condition was variously misdiagnosed as scurvy neonatal osteitis, hemophilia, cortical hyperostosis, leukemia osteitis and congenital syphilis by senior experienced clinicians. The pattern of disorder is minor fracture involving the dia physeal portion of the metaphysis. Extensive subperiosteal hemorrhage occurs and strips the loosed attached periosteum. Squaring and calcifying of the subperiosteal hemorrhage leads to gross deformity of bones which may take years to heal. Multiple deformities are common and may be seriatim rather than concurrent. By a process of exclusion it has been concluded that this pattern may be due either t undue trauma on a normal skeletal system or slight trauma where osseous stability is impaired. The stages of the disease, an abnormal response of normal osseous tissue, are: (1) traumatic damage to the diaphyseal plate, (2) considerable subperiosteal hemorrhage (3) calcification of hemorrhage and squaring of ends of bone (4) bizarre pattern of metaphyseal structure and (5) recovery

W P Sweetnam (Huddersfield) Congenital fibrosis of the liver as familial defect

Congenital cystic disease of the liver is well known. A variant of he condition, congenital fibrosis of the liver is becoming increasingly recognised. In congenital fibrosis of the liver there is an increase of fibrosis in the portal tracts together with an excess of small bile ducts, the zonal pattern being otherwise normal. It seems likely that the progressive dilatation of the bile ducts and the attenuation of the fibrous tissue finally gives rise to the typical polycystic liver There may be an associated polycystic disease of the kidneys (Melnick 1955, Parker 1956) and of the pancreas (Ivemark *et al* 1959) The syndrome may present t birth or may be an incidental finding at autopsy Sherlock (1960) has records of two families in whom all the siblings are affected. This report describes the condition in all three children of one family Biopsy in each case showed typical picture of congenital fibrosis of the liver The first two cases presented at the ages of two and five with hematemesis. The presence of portal hypertension, esophageal varices and portal-systemic collateral circulations were demonstrated by percutaneous transplenic portal venography Both children were protected from further hemorrhage by porta-caval side-to-side anastamoses and have remained well for 6½ and 7 years. One of these children has polycystic kidneys The third child died at the age of six months from pyelonephritis due to polycystic kidneys. It is suggested that congenital fibrosis of the liver is a genetically determined defect and ma be dominant

Diabetes in children and adolescents (Symposium at the Crown Princess Lovisa Chil

dren s Hospital Stockholm). Members of the panel. Y Larsson, G Sterky K M Herrlin, T Möller U Otto and B Person. Moderator Prof. C. Gyllenswärd. The principal part of the symposium will be published in Acta Pædiat.

Meeting Sept 29 1960

W Gaisford (Manchester). Th place of quadruple antigens in immunization

Mult ple antigens are essential in any mass immunization schedule and the advent of poliomyelitis vaccine has enabled the well accepted triple antigen (D P T) to become quadruple There is already ample evidence that these four antigens can be satisfactorily combined. There are two main problems; the first is the earliest age at which immunization can be started with the quadruple antigen. In our studies we have shown that, w th the triple antigen, good results follow after three doses given at 1 5 and 9 weeks of ge if the ant gen contains aluminium phosphate Unfortunately equally good result ha e not followed poliomyelitis immunization at this period of infancy partly because of the inhibitory effect of placen t lly transmitted antibodies and partly use of the poor antigenic property of the elitis Type I component of the accine e second problem is therefore the provi of accine w th a purified and con ent ed T pe I component W ha e been sin such product recently and the results w tai re ues promising three dose pri ary er e is essential t this ge and, with added Type I potency maternally transmitted antibody inhibition can be successfully overcome. The booster response at 1 year in infants receiving three doses of poliomyelitis vaccine at 1 5 and 9 weeks of age is excellent. There is no reason why quadruple antigen should not be given routinely at 1 5 and 9 weeks and a booster at 1 year This would enormously simplify the immunization schedule

In the *discussion* Professor Gaisford said that pertussis was the most important of the four component in earliest infancy and that unfavourable reactions were minimal at this age The risk of pertussis in the first 6 months of lif was likely to be far greater than the risk of complications following immunization and he felt very strongly that pertussis vaccine should be included in all the schedules of early immunization.

Ulcerati colitis n children and adolescents (Symposium at the Pediatric Clinic of Karolinska jukhuset Stockholm). Members of the panel O Broberger L. Billing R. Lagercrantz, H. Rosenqvi t and U Hadbe Moderator Prof. J Lind. Published in part n Acta Pædiat, 49 810 1960.

Meeting Sept 30 1960

J Lorber (Sheffield). The dev lopment of h l oceph l n nfant born with meningo-m locel nd ncephalocel

Ar entric k raph w rmed per for l m comes eries of 11 infant who were born th k ele and encephalocele ket re t e e ncidence of h drocephal s nd facts l progr ion of hese ho had ertal entric la t m earl infancy Th k ele had been repaired surgical emergency soon after admission, mostl in the first week of lif (81 infant or 70%). The entriculograms were performed within 3 weeks of this operation in 69 infants. The verage period between operation and entriculography was pproximately 20 day H drocephalus w found in 94 infant (8) The incidence of h drocephalus w highest in infant hose meningom elocele n ol ed the l mbar region (79 out of 89

(85 %)) in infant with paraplegia (72 out of 8 (9? %)), and in infants whose head circumference at the time of the ventriculogram was over the 90th percentile (61 of 64 (95 %)). The combination of all three of these factors showed that almost all such infants had radiologically demonstrable hydrocephalus in early infancy. The incidence of hydrocephalus in infants whose meningomyelocele did not involve the lumbar region or had no paraplegia was much less, but even in these and even if the head circumference was small or normal, the incidence was still high (57 % and 65 %, respectively). None of the 1 infants who had no hydrocephalus in the neonatal period developed hydrocephalus later. Their mental development was normal, except for the three who had an encephalocele. Repair of the meningomyelocele did not lead to the development of hydrocephalus in any infant. The hydrocephalus, if present, is part of the neurological syndrome at birth and is not related to operative treatment, nor is it a delayed feature although clinical signs may not be present for several weeks or months.

O. H. *Wolff* (Birmingham): Veno-occlusive disease (senecrosis) and protein-losing enteropathy

In August 1958 a 2½ year-old boy developed generalized edema, ascites, pleural effusions and hepatomegaly. The serum albumin was 1.7 g/100 ml; the globulin fractions were also depleted with a gamma globulin of 0.3 g/100 ml. The hypoproteinemia was not the result of a deficient intake of protein. Tests of gastro-intestinal function, barium meal and enema and a jejunal biopsy were normal and malabsorption was thus unlikely. Tests of parenchymatous liver function were also normal and there was no proteinuria. In January 1959 laparotomy and liver biopsy were performed. There was yellowish fluid in the peritoneum, the omentum was edematous, the spleen normal and the liver grossly enlarged with a smooth surface. The histology of the liver suggested the diagnosis of veno-occlusive disease. The centrilobular veins were partially occluded by loose collagenous reticulum in their walls. The surrounding sinusoids were dilated and engorged with blood. It then came to light that the patient was in the habit of eating various plants, his favorit being groundsel (*Senecio vulgaris*). With the object of preventing the development of cirrhosis—a recognised complication of veno-occlusive disease—the patient was given a course of prednisolone. The effect was surprising: within a week of starting prednisolone in a dose of 25 mg daily the serum albumin had risen from 2.2 to 4 g/100 ml. The globulin fractions took 3 weeks to return to normal. There was a quantitative relationship between the dose of prednisolone and the albumin level, so that when the dose was reduced to 5 mg daily the albumin fell to 3.5 g and when the dose was increased to 10 mg the albumin rose again to 4 g/100 ml. After 3 months prednisolone was discontinued, the edema reaccumulated and steroid therapy had to be restarted. In March 1960 a repeat liver biopsy showed great improvement; the centrilobular veins were normal and there was only slight distension of the sinusoids. While still on prednisolone the patient was given an intravenous dose of I[131] labelled polyvinylpyrrolidone (P.V.P.). At the time of this test the serum albumin was 4 g/100 ml. Only 1 of the dose of P.V.P. appeared in the feces, this is a normal result. Prednisolone was then discontinued and when the albumin level had fallen to 2.5 g/100 ml a second dose of P.V.P. was given and 12.5 of the dose appeared in the faeces. Thus gross leakage of protein into the alimentary tract was considered to be responsible for the hypoproteinemia. The response of the protein-losing enteropathy to steroids reminds one of the situation in nephrosis where the protein leak through the glomerulus responds similarly. The relationship between the protein losing enteropathy and the veno-occlusive disease remains obscure. It is conceivable that some of the unexplained cases of protein-losing enteropathy will be shown to be instances of veno-occlusive disease (senecrosis). However

another and possibly more likely explanation is that this patient suffered from primary protein losing enteropathy and that the constant drain of protein predisposed his liver to the toxic action of the pyrrolizidine alkaloid in the groundsel.

Hemolytic anemias in the Pediatric age group (Symposium at the Pediatric Clinic of Akademiska sjukhuset, Uppsala). Members of the panel: L. Garby C. Högman, A. Källander S. Sjölin and C. de Verdier Moderator: Prof. B. Vahlquist. Will be published in Acta Pædiat, 1961.

Meeting Oct. 14 1960

B Hellström Aspects of th diagnosis of rheumatoid arthritis in children (to be published in Acta Pædiat)

H Herzenberg and V Eskeland The morphological development of pulmonary arteries during the first years of life

In order to show the normal morphological development of intrapulmonary arteries during the first years of lif lung preparations from 66 children ranging from birth to 5 years were examined microscopically These children had died of disease not primarily affecting the respiratory or circulatory organs. The vessels were classified in four groups according to size and structure. Dia- of the lumen and thickness of the vessel were measured and the ratio between two was calculated. At birth and -eral days thereafter the pulmonary -rterial bed has a characteristic appearance with narrow luminae and thick vessel walls. The ratio is low During the first months of life the lumen enlarges and the vessel wall decreases in width, resulting in an increased ratio *This change takes place more slowly* during the first weeks of life than later on. This helps to prevent a large left-to-right shunt through an open ductus which might result in left ventricular failure. At heart catheterization and angiocardiography a left-to-right shunt through an open ductus has been shown to exist up to two weeks of age. As soon as the ductus has closed, the widening of the lumen and the thinning of the vessel wall occurs more quickly After the fourth month of life this transition occurs more gradually and by the eighth month of life has been largely completed By then, the vessels of the lungs resemble those characteristic of the adult in whom the pulmonary vascular bed is adjusted to a circulation with low resistance.

P Zetterqvist Familial atrial septal defects

Data on several cases of atrial septal defect of the secundum type in one family is presented. The pedigree suggests dominant mode of inheritance in four generations with eight certain and five suspected cases. In four cases the diagnosis was confirmed by anatomical inspection of the heart three at the time of surgical correction. Cytogenetic studies have shown chromosomal aberrations varying in type in different individuals. These findings are similar to those reported in mongolism and suggest caryotypic and probable pathogenetic differences. In healthy member of the family an aberration in the form of a balanced translocation has also been found. The pathogenesis of chromosomal berrations is discussed.

L. Gustafson, R. Lagercrantz d A Lagerdahl Sleep disturbances in infancy and early childhood

A child psychologist a pediatrician and a public health nurse collaborated at the Norrtull Well Baby Clinic in Stockholm Every fourth mother to children between the ages of 9 months and 4 years was asked if her child had presented sleep disturbances which had troubled or disturbed the family Ninety three mothers were asked and 89 answered. Twenty-two (25%) stated that their children had had insomnia for at least 4 weeks during

the preceding 6 months. Sleep disturbances were specially common below the age of years. Difficulties in falling asleep were more common among boys than girls. The latter awakened more often at night. Most children had had insomnia for more than 7 months. Children with sleep disturbances were compared with those without in the initial sample as well as with a selected material of social twins without sleep disturbances. The comparison was based on the results of interviews with mothers whose relationship and attitude to their children was graded. The results were analyzed statistically primarily with the "sign test". Social status, family situation or lodging was not found to have any significant effect on the incidence of insomnia. A more or less significant relationship was established between insomnia in the child and the following: feeding difficulties, undue liveliness in the child, anxiety in the mother and inconsistent handling of the child. Several factors such as *feeding difficulties, frequent infections*, tiredness in the mother and inappropriate attitude toward the child were often present in the same family. Collaboration between child psychologist, pediatrician and public health nurse was found to be of significant value in the handling of these cases.

R. Lagercrantz, Stockholm

Erratum

In order to avoid misinterpretation it should be noted that the word *rubeola* in the report of the Swedish Pediatric Society's proceedings of Feb. 12, 1960 (Acta Pædiat 49: 453, 1960) means *German measles* (*rubella*).

humanitarian aspects on the medical profession and his great common sense. The reviewer wants to call especial attention to those readers who do not know the important achievements of Sir James and for whom he is only one of the famous names in the history of pediatrics in Europe, to study carefully the f llowing articles: Nature of Disease, Children and Families, Care of Children in Hospitals and the Need for Understanding the Individual. It is most fortunate that the Editors hav accomplished this collection of Professor Spence s papers, thus facilitating references, even to articles that originally were published in journals that are not easily accessible.

One reads with great pleasure the vivid picture of Sir James by Sir John Charles in his introductory 26 pages biography in which are included some written souvenirs of Spence's friends. His successor Professor Donald Court, call him "artist and scientist, romantic and realist, conservative and rebel, always a leader" He was without doubt *one of the great physicians of this century* He has contributed much to the advancement of our knowledge in pediatrics, especially its social implications. He was a rarely ...ed man, who used his own way in ...ng at problems and in trying to solve ... and in developing the science and art of being a physician for children. This book should be included in every private pediatric and official library

J F de Wijn and J H de Haas Growth Diagrams from 1–25 Years in th Netherlands.

Nederlands Instituut voor Praeventieve Geneeskunde Leiden. 1960 20 pages. Price fl. 2.50

This comprehensive report is written in Dutch but an English summary and English translation of the text of the 11 growth curves facilitate the reading The study is a cross-sectional one of the height, weight and secondary sex characteristics of 8796 boys and 8112 girls of average social class. The averages are at a higher level than in former times and it is believed that in the near future the children will be taller and heavier than the children of today It is planned to complete this investigation with a longitudinal study

Moshe Prywes (ed.) Medical and Biological Research in Israel.

Rubin Mass, Jerusalem, Israel. 1960. 562 pages, numerous illustrations. Price $6.

This nice volume is divided into eleven sections of which the sixth (Experimental and Clinical Research in Medical Disciplines) contains two pages related to research in pediatrics. Report is given of studies in malnutrition and diarrhea (electrophoretic patterns of serum proteins, serum levels of *vitamin A, intestinal* flora and plasma prothrombin level), of diseases of the heart (incidence of congenital and rheumatic heart disease, an outbreak of Fiedler interstitial myocarditis, Coxsackie-virus as cause of heart disease) and of the newborn infant (early jaundice due to ABO incompatibility evaluation of the state of maturation of the nervous system by means of studies of the reflexes). The reader is impressed by the great activity and high standard of research in Israel.

T Yoshida and J Huizinga Abstracts of Japanese Medicine.

Excerpta Medica Foundation, Amsterdam, 1960. Annual subscription rate $30.

A new journal reviewing about 500 current Japanese periodicals has just been published. The pediatric part of the journal, which in this first issue contains eight of the total 187 pages, is divided into 23 different sections, covering all items within the pediatric field f medicine. Acta Paediatrica wants to draw the attention of its subscribers to this new service of Excerpta Medica.

Acta Pædiatrica 50: 217—227 May 1961

Institute for Medical Genetics, University of Uppsala, and Pediatric Clinic Karolinska Jukhuset Stockholm, Sweden

Association Between Congenital Heart Malformation and Chromosomal Variations[1]

by J. A. BÖÖK, BERTA SANTESSON and PER ZETTERQVIST

Knowledge of chromosomal variations in man as a cause of abnormal development and disease has increased rapidly during the past two years. At the present time about twenty five conditions associating numerical or structural chromosomal variations and pathological conditions have been reported about half of them concern the sex chromosomes and the rest the autosomes. Even if some of the observations are still represented by single individuals only there is no doubt that clinical cytogenetics, in a very short time has been established as a new and important branch of human pathology and clinical medicine.

Practically all individuals on record who lacked one chromosome e.g. X0 in Turner's syndrome (8) or who had an additional chromosome such as trisomic 1 in mongolism, trisomic [16–18] in Harnden Patau's syndrome (7 11) and trisomic [13–15] in Patau's syndrome (11) have shown a multiplicity of developmental and morphological changes.

Exceptions from these findings of multiple changes are two individuals with congenital heart defect (1 5) who will be dealt with in some detail in this paper and an apparently healthy father of a child with mongolism reported from this laboratory by Fraccaro *et al.* (9). This man, who had 47 chromosomes, might be trisomic [19–20], but unfortunately no clinical examination was made

Malformations of the heart have been a frequent feature of the "chromosomal" syndromes mentioned above This and the fact that one of us (Zetterqvist) has been particularly interested in pediatric cardiology was the reason why we started to collect information on the karyotypes of individuals with congenital heart malformations.

One of us (Zetterqvist (13)) has reported an exceptional pedigree with eight certain and five probable cases of atrial septal defect of the secundum type The distribution of affected and normal individuals in this pedigree appears to agree with a simple dominant mode of inheritance We have now been able to study the karyotypes of one family belonging to this pedigree

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Aided by grants from the Swedish Atomic Research Council and the International Atomic Energy Agency

[1] The Denver nomenclature of human mitotic chromosomes (6) will be used consistently in this paper (cf. Fig 1). The expression trisomic is used for brevity although it is noted that the diagnosis is based on the morphological similarity of mitotic chromosomes only

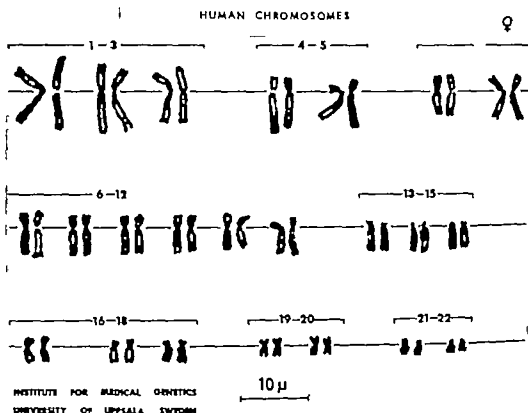


Fig 1 The normal female chromosome set arranged in accordance with the Denver agreement.

Description of the family

The mother E. G was aged 49 (No. III:6 in Zetterqvist (13) original pedigree) She has had dyspnea on moderat exercise since childhood. Her condition deteriorated in 1957 following an attack of influenza. At the special examination in October 1959 which included catheterization and angiocardiography a large auricular septal defect of the secundum type was found. This defect was associated with mitral insufficiency due to relative dilat tion f the mitral ring without cusp malformations. The relative heart volume was 900 ml per sq.m body surface area. The blood flow through the pulmonary and systemic circuits was 34 and 4 l per minute, respectively Both malformations were corrected at an operation in November 1959 at the Chest Clinic in Stockholm (Professor C. Crafoord, M. D). The postoperative course was uneventful and the patient is now (June 1960) in good health.

Cytological observations on E G

Method. Chromosome studies were made according to the technique developed in this laboratory (3). The cytological observations on the patient E. G are based on four separate cell cultures from bone marrow biopsy taken Oct. 21 1959, and four separat cultures from a skin biopsy taken January 15, 1960. At the preparation of the skin biopsy two cultures only were started from trypsinized material. The other two

TABLE 1 *Summary of chromosome findings in a family in which mother and son had an atrial septal defect of the secundum type.*

	Individual and origin of cultured cells	Chromosome counts 45	46	46±1	47	Total number of analysed cells	Chromosome complement
E.G.	Mother bone marrow	4	4	0	25	33	Trisomic [19–20]; XX
	Mother skin	2	6	0	17	25	Trisomic [19–20]; XX
C.G.G	Father bone marrow	0	8	0	0	8	Normal; XY
C.M.G	Son, bone marrow	1	19	1	0	1	Trisomic [19–20]; Monosomic 22 XY
	Son, skin	0	10	1	0	11	Trisomic [19–20]; Monosomic 22; XY
I.G.	Daughter bone marrow	0	9	0	0	9	Normal; XX

were prepared by mincing the material directly in the medium with pair of fine ophthalmological scissors. This process is apparently less damaging to the cells. By omitting the initial trypsinization for skin, muscle and fascia lata biopsies the time lag between the preparation of the culture and the first signs of growth has been shortened to an average f 14 days for adult material.

The bone marrow cultures have been observed over a period of 2 months. Microscopic slides were prepared from primary as well as trypsinized and transferred cultures. The skin cultures have been studied in the same way for a period of bout three months. As the results from the examination of the different cultures and subcultures were all consistent they will not be itemized. W have carefully selected and examined as pparently undamaged cells in mitotic metaphase derived from the skin and bone marrow of E. G

The result of the chromosome counts have been summarized in Table 1 The majority of the cells contained 47 chromosomes but by our standards there were too many quite clear metaphases with 46 to be accounted for by technical biases.

Five 47 chromosome cells derived from the bone marrow and three derived from the skin have been analysed in detail by the aid of enlarged photomicrographs. In all these cells we found five short chromosomes with nearly median centromeres. Thus, on morphological grounds the additional 4 th chromosome was classified as belonging to group [19–20] In Fig 2 which exemplifies the karyotype this additional chromosome has been placed at position 19 Tentatively these cells were diagnosed as trisomic [19–20].

Two 46 chromosome cells were likewise analysed in all details. Matching of the chromosomes showed a normal complement with two X-chromosomes. Also in the remaining 46 chromosome cells only four short chromosomes with nearly median centromere could be recognized.

The 45 chromosome cells (cf. Table 1) displayed no consistent chromosome pattern and are most reasonably explained as due to the accidental loss of one chromosome when the squash preparations were made

The husband, C. G G born in 1910, is a normal healthy male without signs or symptoms of cardiac disease. There is no consanguinity between him and his wife.

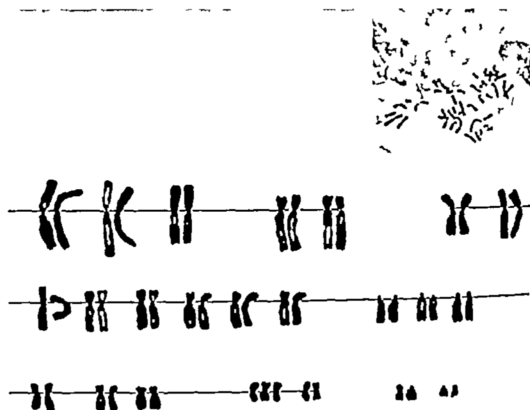


Fig. 2. The chromosome complement of the mother with congenital heart defect, apparently trisomic [19–20].

Eight carefully analysed cells in mitotic metaphase, derived from three separate bone marrow cultures, showed a normal male complement with 46 chromosomes, as exemplified in Fig. 3. This couple has two children, a boy born in 1948 and a daughter born in 1950. There are no further issues and no abortions.

The boy C. M. G., on routine examination was found to have signs of heart disease, although he had no subjective complaints. At catheterization and angiocardiography an atrial septal defect of the secundum type was disclosed (i.e. the same defect as in the mother). An operation was not considered necessary as the pulmonary/systemic flow ratio was shown to be 1.5/1 only.

Cytological observations on C. M. G.

The observations are based on three separate cell cultures from a bone marrow biopsy and two separate cultures from a skin biopsy, both taken on January 15, 1960. Both sets of cultures have been observed over a period of about three months. The results from the primary as well as the transferred cultures were all consistent. Thirty two cells in mitotic metaphase have been analysed. The results

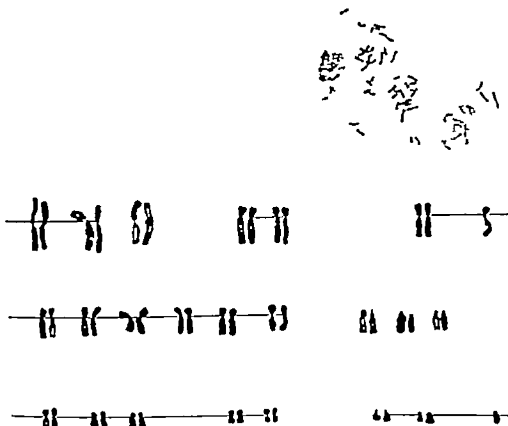


Fig. 3. The chromosome complement of the father pparently normal.

of the chromosome counts have been summarized in Table 1 Practically all cells in which exact counts could be made (20 out of 30 cells) contained 46 chromosomes. In 15 of these cells with especially clear met phases four small acrocentric chromosomes only were found gainst an expected five in a normal male Further more there were five hort chromosomes with median or nearly median centromere (group [19–20]) against an expected four

Three cell derived from the bone marrow and four from the skin have been analysed further by the aid of enlarged photomicrographs. All these cells contained a fifth short chromosome with median or nearly median centromere Le apparently the same constellation as in the mother One small acrocentric chromosome was lacking As in several cells two small acrocentric chromosomes with satel lites (no. 21) and the Y-chromosome could be recognized, it is possible that one chromosome number 22 was lacking As ex

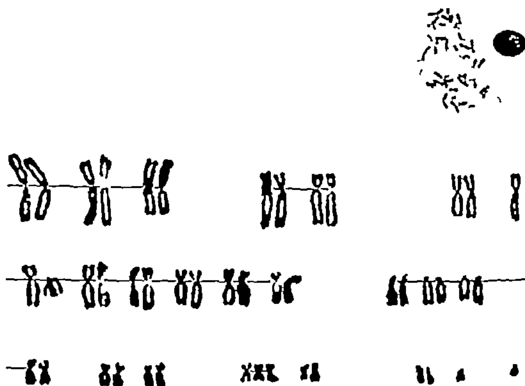


Fig. 4. The chromosome complement of the son with congenital heart defect apparently trisomic [19–20] and monosomic 22.

emplified in Fig. 4, the karyotype of this individual could be interpreted as trisomic [19–20] and monosomic 22.

The daughter I. G., was healthy and showed no signs or symptoms of cardiac disease on physical examination, including electrocardiography. A detailed analysis of the chromosome complement in cells from three separate cultures of a bone marrow biopsy showed morphologically normal conditions, as exemplified in Fig. 5.

The diagnosis of individual karyotypes

It is known that cells cultivated *in vitro* over long periods may change their karyotype. This is presumably due to a number of different factors operating in a highly artificial environment. However a considerable amount of the experience with human cell cultures comes from work with malignant cells which originally may consist of a mixture of different karyotypes. The work in this laboratory has been limited to the study of individual karyotypes with the exclusion of all kinds of malignant cells. In laboratories where malignant cell strains are maintained the possibility of infection from such cultures must be considered.

We have found, as have others (12), that open system fluid medium cultures

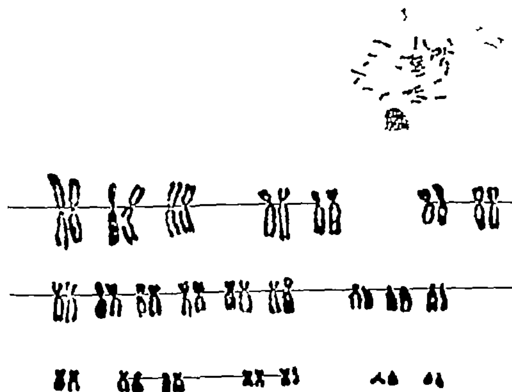


Fig. 5. The chromosome complement of the daughter apparently normal.

favour karyotype constancy for periods which are long enough to secure a sufficient margin of safety for karyotyping. This has been established in our laboratory during the work with more than 400 cultures from biopsies of about 1°0 individuals and over 100 cultures derived from human fetuses. In several instances the cultures have been checked for the constancy of the karyotype for periods up to six months. In general the number of polyploid cells with multiples of the diploid number tends to increase after several transfers. Usually this will not interfere with a correct interpretation but may even be a help to establish the identity of additional chromosomes. Occasional aberrations have been observed, e.g. chromatide breaks, somatical pairing and anaphase disturbances. The incidence of such abnormalities has been estimated not to exceed one per cent of the mitoses and the disturbances did not increase significantly during the time the cultures have been observed. Nevertheless it is recommended that, whenever possible karyotyping be made on early cultures and preferably on the primary ones. Minor modifications of the culture medium or technique appear unimportant in this context. A number of independent laboratories using different techniques has now

provided sufficient data on normal karyotypes and on the common karyotype in mongolism and Turner's syndrome to prove this point.

It cannot be avoided that the selection of a particular metaphase for analysis is subjective and depends on the experience and judgement of the individual cytologist. Certain criteria are used to decide whether or not the squash has damaged the cell to such an extent that a chromosomal analysis is pointless. Our procedure is as follows: using a magnification of ×160 we select apparently undamaged metaphases with good, even spreading of the chromosomes, care being taken that there are no other cells in the same mitotic stage in the vicinity. Using this method we have found it to be of questionable value to increase the number of metaphases by pretreatment with colchicine or colcemid. Furthermore, with our process we are more likely to include occasional metaphases in which one chromosome has been lost than those having received an additional chromosome dislocated from a nearby metaphase.

With the cell culture technique it is not possible to prove directly that the karyotype determined for the cells *in vitro* is exactly the same as in the donor's body. The findings must be corroborated by circumstantial evidence. The general process in this laboratory is to start 3–5 independent cultures from each biopsy and determine the karyotype on the primary as well as the transferred cultures over a period of 2–3 months. While it is possible that new karyotypes may arise e.g. through mitotic non-disjunction, it is extremely unlikely that the same mutation will occur in all the primary and transferred cultures. No conclusion regarding the karyotype of the donor will be drawn unless the findings in all cultures are consistent. Furthermore, for conclusions concerning a new pathological karyotype we require consistency with regard to cultures derived from at least two different biopsies, usually bone marrow and skin. When inconsistencies are observed the whole procedure will be repeated.

The question of mosaicism presents particular problems. Few cases appear as clear as an individual (4, 5) who apparently is a 3n/2n mosaic where all cells so far derived from the bone marrow were diploid. The usual finding, as in the mother of the family described here, is a mixture of cells with a normal complement and aberrant cells. Trisomic cells, for example, may have a tendency to eliminate the additional chromosome. If so, consistency among the different primary and transferred cultures will not prove the point. There is no way of knowing whether the alleged elimination, if at all occurring *in vivo*, takes place more rapidly *in vitro*. It seems doubtful if karyotyping based on the direct incubation of bone marrow cells will help much. In so far as mitotic activity is concerned the bone marrow is most similar to our *in vitro* cell cultures. Consequently, chromosomal aberrations which tend to interfere with the mitotic mechanism could be more heavily selected against here than in other tissues. It should also be kept in mind that a large number of drugs at therapeutic concentrations and industrial or professional chemicals are known to affect the bone marrow in particular. These effects may have a different selective value on dif-

ferent karyotypes as well as being instrumental in creating chromosomal abnormalities.

Discussion

From the data referred to above it seems reasonable to infer that the karyotypes of the cultivated cells correspond to those of the donors. By morphological criteria the 47th chromosome of E. G is similar to the additional short chromosome with nearly median centromere in her son.

The lack of one small acrocentric chromosome in the son is remarkable particularly in the absence of gross effects. The very consistent karyotype findings in the cells derived from this individual, make it unlikely that we are dealing with an artefact.

The son, C. M. G., would have originated through an egg cell containing an additional chromosome [19–20] About 50 of the mother's egg cells would be expected to have this constitution The same egg cell may due to non-disjunction, have lost chromosome number 22 Possibly the extra 47th chromosome might be a disturbing factor t meiosis Alternatively the 4 chromosome egg cell might have been fertilized by a sperm with 22 chromosomes only and lacking number 22 due to non-disjunction in the father

The origin of the abnormal karyotype of the son could also be explained by a mechanism of translocation involving chromosome number 22. If so the chromosome interpreted as an extra [19–20] in Fig 4 might be a 22/[10 20] translocation. Such a translocation could have resulted in a new chromosome indistinguishable from the other members f group [19–20] Then this alternative implies a partial deficiency of one chromosome number 22 and a partial triplication of the gene material contained in the chromosome of group [19–20].

On morphological grounds alone it is not possible to give priority to either of these two explanations. Both would be consistent with an association between triplication of gene material of the chromosomes of group [19–20] in mother and son if not with simple trisomy

Several other alternative explanations involving translocations or combinations between translocations and non-disjunction are of course theoretically possible. On the basis of now available evidence those offered here seem to us the most likely ones.

This communication should not convey the impression that we consider congenital heart defects in general are due to chromosomal aberrations. Nevertheless, in view of the fact that such defects form a significant feature of several chromosomal syndromes, among which mongolism is the most common, this etiological mechanism appears important in cardiac pathology

We have been looking for chromosomal aberrations in a few other patients with congenital heart defects but with negative results. Two of them have now been karyotyped and merit mention briefly

File no. 01–45–60, L. M. A., female born Feb. 28, 1960, died March 27 1960 Maternal age 27 paternal age 29 She had one normal brother born in 1954 and one normal sister born in 1957 There were no abortions. The pregnancy was uneventful and the birth weight 3840 gram. She was blue baby with permanent heart incompensation. The post mortem examination showed deformities f the tricuspid and mitral valves, pronounced hypertrophy and dilatation of the

right ventricle, dilatation of the right auricle and a patent ductus arteriosus. A detailed analysis of the chromosome complement in four separate cell cultures derived from a skin biopsy taken March 12, 1960 showed morphologically normal conditions, i.e. 46 chromosomes including two X-chromosomes.

File no. 01 61-60 S. G. L., male, born Feb. 16, 1951. Maternal age 22, paternal age 4. H. had scarlet fever in 1955. At this time a heart murmur was detected. An atrial septal defect of the secundum type was confirmed by electrocardiography, catheterization and angiocardiography. In addition he had multiple minor skeletal deformities of the shoulder girdle and hands. As the blood flow of the pulmonary circuit was more than twice that of the systemic circuit an operation was considered indicated and also performed in 1959. The karyotyping was based on three separate primary cell cultures derived from a bone marrow biopsy taken March 22, 1960. H. was found to have an apparently normal male complement.

This patient is particularly interesting because his mother had a similar defect. She was M. H., born in 19?9 and had deformities of the upper extremities similar to those in her son, only more marked, and with total absence of her right thumb. On the left hand the thumb was substituted by a small radial finger and the thenar part of the hand was malformed. The electrocardiogram showed marked arythmia of an unusual type with extreme sinus bradycardia, frequently interrupted by sino-auricular block and an irregular nodal tachycardia. The ventricular complexes, however, were of the type usually seen in atrial septal defect of the secundum type. X-ray findings practically confirmed the existence of a left-to-right shunt at the atrial level. So far she has not been available for karyotyping.

The etiology of congenital heart defects is apparently manifold and complex ( ). Abnormal chromosome complements may be instrumental in some instances, while gene mutations or external factors operate in others. The findings reported in this paper indicate the necessity of paying further attention to chromosomal variations and the mechanisms lying behind these. It is also obvious to us that further confirmatory work is needed. It is hoped to subject further members of the pedigree to cytogenetic studies later.

As the incidence of meiotic disturbances resulting in numerical and/or structural variations are known to increase with increasing doses of ionizing radiation (10), the question of further human defects due to chromosomal aberrations and at the same time compatible with appreciable fertility merits a particular interest. So far the genetical effects of ionizing radiation on human populations have been thought of almost exclusively in terms of gene mutations. With the present knowledge of at least twenty-five different chromosomal variations apparently causing defects in a substantial number of individuals, the association between these phenomena and different levels of ionizing radiation must be given serious consideration.

Summary

A family is described in which mother and son both displayed auricular septal defects of the secundum type while father and daughter had no cardiovascular disorders. The karyotypes of these individuals were analysed by the cell culture method using biopsies from bone marrow and skin. The majority of the cells from the mother had 47 chromosomes and she was apparently trisomic [19–20]. A similar additional chromosome of group [19–20] was found in the cells derived from the son.

However he lacked one of the smallest acrocentric chromosomes. His chromosome number was 46 The father and daughter both had normal karyotypes. The significance of numerical and/or structural chromosome variation in the etiology of congenital heart malformation is discussed with regard to recent developments in clinical cytogenetics.

References

1. Böök, J A.: Mongolism and other conditions associated with chromosomal variations. *Acta Soc Med Upsal* *65* 1 2 1960.
2. — Heredity and heart disease. *Amer J Publ Health*, Part II, *50* 1–4 1960.
3. Böök, J A., Fraccaro, M. and Lindsten J Cytogenetical observations in mongolism. *Acta Paediat* (Ups), *48* 453–468, 1959
4. Böök, J A. and Santesson B.: Malformation syndrome in man associated with triploidy (69 chromosomes). *Lancet* *I* 858–859 1960.
5. — Further studies in clinical cytogenetics. *Acta U lo Lund*, *Ser 2* *56*: 19–20, 1960.
6. Böök, J A. *et al* A proposed standard system of nomenclature of human mitotic chromosomes. *Lancet* *I* 1063–1065, 1960.
7. Edwards, J H., Harnden D G Cameron A. M., Crosse, M. V and Wolff O H.: A new trisomic syndrome. *Lancet* *I* 787–790, 1960.
8. Ford, C. E., Jones, K. W Polani, P E., Almeida, J C. de and Briggs, J H A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome) *Lancet* *I* 711 1959
9. Fraccaro, M., Kaijser, K. and Lindsten J.: Chromosomal abnormalities in father and mongol child. *Lancet*, *I* 724–727 1960.
10. Kaufmann B P Chromosome aberrations induced in animal cells by ionizing radiations. *I* Radiation Biology ed. A. Hollaender McGraw Hill, New York, Toronto, London. Vol. *I* 627 711 1954.
11. Patau K.: The identification of individual chromosomes, especially in man. *Amer J Hum Genet*, *12* 250–277 1960
12. Tjio, J H. and Puck, T Genetics of somatic mammalian cells. II. Chromosomal constitutions of cells in tissue culture. *J Exp Med* *108* 259–268, 1958.
13. Zetterqvist P Multiple occurrence of atrial septal defect in a family *Acta Paediat*, (Upps.) *49* 741 1960

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As the incidence of meiotic disturbances resulting in numerical and/or structural variations are known to increase with increasing doses of ionizing radiation (10), the question of further human defects due to chromosomal aberrations and at the same time compatible with appreciable fertility merits a particular interest. So far the genetical effects of ionizing radiation on human populations have been thought of almost exclusively in terms of gene mutations. With the present knowledge of at least twenty-five different chromosomal variations apparently causing defects in a substantial number of individuals, the association between these phenomena and different levels of ionizing radiation must be given serious consideration.

Summary

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The relationship between the muscle mass and the output of the heart in the foetus is not certain. Dawes, Mott, Widdicombe & Wyatt (5) suggested that in lambs the left ventricular output exceeded the right ventricular output throughout foetal life and Keen (11) suggested that the same occurs even in the full-term infant heart which shows anatomical right-sided preponderance. The mean muscle fibre diameter of the right heart is greater than the left heart in the foetus (2). Boellaard (4) followed Ashley's work of measuring thickness of the muscle fibres. He showed that there was possibly some slight thinning in the total thickness of the right ventricular wall but any possible thinning of the diameter of the fibres did not account for the apparent "atrophy" and he postulated that the changes after birth were probably chiefly those of dilatation of the cavity. His material and method of assessment are subject to many of the same criticisms as that of Keen's material.

There is very great variation in the relative left and right heart weights at birth and so many factors are involved in the study of such hearts that confusion can only be prevented by separating the various criteria. Emery & MacDonald (6) have shown that analysing the same series of children's hearts against crown–rump length, gestation age and weight of the child, produces a different growth pattern. By means of careful dissection of the left and right ventricles in a series of stillborns, they were able to establish what they felt were the normal weight increments of the cardiac ventricles against gestation age, crown–rump length and body weight for the stillborn child during the latter half of gestation. In dealing with postnatal changes it is obvious that body weight can hardly be used as a criteria for analysis due to the great variability in postnatal change in weight and even such variables at birth as inhalation and the passage or not of much meconium.

In the present study an attempt has been made to compare the actual weights of the cardiac ventricles in children that had lived for more than 5 days after birth with the weights of stillborn children of the same crown–rump length. Any difference in these two findings would suggest either an increase or decrease in the immediate postnatal rate of growth of the heart muscles.

Material and Methods

A careful selection was made of the post mortem reports to find children who had been born before 38 weeks gestation and who had lived for 5 days or more, and in whom there was no gross congenital deformity and no evidence that the child had died directly of circulatory disease. Eighteen such cases were found after surveying approximately 1500 necropsies during which time all children's hearts had been saved. Eight cases had a crown–rump length of between 25 and 29.5 cm and 10 between 30 and 34.5 cm.

In this study we deliberately excluded children dying between birth and five days for two main reasons. One is that during this period the causes of death are too difficult to be stated with any certainty and the other is that it is the effect of postnatal life that is required, and it would not be certain whether any changes could take place of any significance within two or three days of birth.

These hearts were dissected and the ventricles weighed using the same method as that previously described for stillbirths (6), which method is similar to that used by Hermann & Wilson (8) and Panisello, Castellanos, Junco & Valladares (17). The data from these cases were compared with those of the apparently normal stillbirth series.

Comparison was made between the groups of equivalent crown rump length and t tests were applied according to the formula: Difference between means/Standard error of difference between means. The values thus obtained for each of the groups were then related to the values obtained from n number of cases located in t tables at 0 05 levels. The n numbers used being $(n_{1\ 1})$ and $(\ _{2-1})$ for each comparable group.

Results

The individual ventricle weights related both to crown–rump length of the child and to the mean line of the values obtained from stillborn children are shown in Fig 1 It is obvious that the scatter values in the right and left ventricle charts is different In the case of the right ventricle the values appear to be evenly distributed around the mean line whereas in the left ventricle the majority are above the line The statistical analyses of the means of these groups are presented in Tables 1 and 2. These show that there is no significant difference between the right ventricular weights of the stillborns and children who lived after birth, of comparable crown-rump length. On the other hand there is a significant difference (at the 95% and even at the 99% level) between the left ventricular weights of the "living" and stillborns. This finding would suggest that within five days after birth there has been an appreciable increase in the weight of the left ventricle over that which would have occurred had the child not been born i.e. significant postnatal hypertrophy

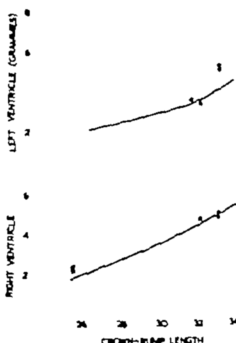


Fig. 1 Showing the weights of the left and right ventricles (shown as spots) against the mean line of a series of stillborns of the same crown-rump length.

With regard to the right ventricle there is no such deviation from the apparent intrauterine growth pattern Had there been any postnatal actual atrophy or

TABLE 1 *Showing the numbers of cases studied and the mean values obtained together with standard deviation in two crown–rump length groups*

Crown–rump length (cm)	Stillbirths (J.L.E. & M.S.M.) No. of cases ( )	L.V wt. (g) Mean	L.V wt. (g) S.D	R.V wt. (g) Mean	R.V wt. (g) S.D	Livebirths No. of cases ( )	L.V wt. (g) Mean	L.V wt. (g) S.D	R.V wt. (g) Mean	R.V wt. (g) S.D
23–29.5	70	4	0.68	2.6	0.58	8	3.90	1 16	[illegible]	[illegible]
30–34.5	45	3.7	[illegible]	4 4	2.04	10	5.37	1 42	4.73	1.17

TABLE ... *Showing the* t *values obtained against* t *values from tables at 1 in 20 probability*

Crown-rump length (cm)	Left ventricle		Right ventricle	
	t value obtained	*t* from tables*	*t* value obtained	*t* from tables*
25–29.5	2.72	2.056	1.44	2.064
30–34.5	3.42	2.01	0.729	2.01

* At 0.05 level.

hypoplasia of this ventricle during this period as postulated by Hort (9) and Keen (10) we would have expected to see lower mean weights in the postnatal ventricles. It may be argued that the postulated atrophy had not had time to commence in our material but the positive find of hypertrophy of the left ventricle in the same hearts does suggest that a change in the opposite direction, if present would have been revealed.

We feel justified in suggesting that there is no evidence for postnatal atrophy of the right ventricle.

Summary

The mean ventricular weights of prematurely born infants who had lived for five or more days were compared with the mean ventricular weights of stillborns of equal crown rump lengths. The left ventricular weights suggest that there has already been some hypertrophy over that which would have occurred *in utero*. The right ventricle growth continued at the same rate as would have occurred had the child remained *in utero*.

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References

1. ALIMURUNG, M. M., JOSEPH, L. G., NADAS, A. S. and MASSELL, B. F.: The unipolar precordial and extremity electrocardiograms in normal infants and children. *Circulation*, 4: 420–429, 1951.
2. ASHLEY, L. M.: A determination of the diameters of ventricular myocardial fibres in man and other mammals. *Amer J Anat*, 77: 325–359, 1945.
3. BARCLAY, A. E., FRANKLIN, K. J. and PRICHARD, M. M. L.: The Foetal Circulation. Blackwell, Oxford, 1944.
4. BOELLAARD VON J. W.: Über Umbauvorgänge in der rechten Herzkammerwand während der Neugeborenen, und Säuglingsperiode. *Z. Kreislaufforsch*, 41: 101–111, 1952.
5. DAWES, G. S., MOTT, J. C., WIDDICOMBE, J. G. and WYATT, D. G.: Changes in the lungs of the newborn lamb. *J Physiol*, 121: 141–162, 1953.
6. EMERY, J. L. and MACDONALD, A. M.: The weight of the cardiac ventricles during the later weeks of intrauterine life. *Brit Heart J* (in press).
7. FULTON, R. M., HUTCHINSON, E. C. and

TABLE 1 *Summary of the recent literature on the iron and copper concentration of the liver of the foetus and newborn*

Author	Number of cases	Age group	Iron: Wet liver mg/100g	Iron: Dry liver mg/100g	Copper: Wet liver mg/100g	Copper: Dry liver mg/100g	Comments
Gladstone (7)	17	Stillborn, 1 day	9.9–30.8				Non-haemoglobin iron
Schwartz, Baer & Weiser (16)							Similar findings to Gladstone
Ramage, Sheldon & Sheldon (14)	6	Below 24 weeks gestation		210			Total iron
	6	Full term		250			
Toverud (19)	47	Full term		200			Method includes some haemoglobin iron
	53	26–35 weeks gestation		168			Estimations on combined liver and spleen
Iob & Swanson (9)	14	Foetuses	19.4–72.7				Total iron
Brückman & Zondek (1)	5	Newborn	8.8–58.5			8.0–38.2	Non-haemoglobin iron
Widdowson & Spray (20)	11	Immature and stillborn	6.8–37.0				Inorganic iron
	14	Immature and stillborn			1.83–8.70		Estimations on combined liver and spleen
Smith, Rosello Say & Yeya (18)	14	0–6 months	6–16				Total iron
Kaldor (10)	6	Stillborn over 3150 g	Over 50				Non-haemoglobin iron
	10	Stillborn under 3150 g	Under 50				
Morrison & Nash (12)	35	Infants			2.4		
Sheldon & Ramage (17)	5	Foetuses				15–35	
Butt, Nusbaum, Gilmour & Didio (2)	46	Premature				38.5	Includes children up to 3 months after birth
	34	Mature				20.49	

fixed as distinct from fresh livers. It was found that fixed tissues gave slightly lower results

The copper estimations were carried out on parallel samples using the method of Eden & Green (5)

Each liver was treated as two separate livers, the right and left lobes being treated independently. Thus the total number of specimens examined was 150 from 75 children who had shown no Rh incompatibility and in whom no gross abnormalities of the liver were noted either by naked eye or on examination of sections microscopically. Livers from 11 children who had been suffering from known Rh incompatibility were also examined.

Results

The general results of the chemical analyses are presented in Table .. Different aspects of the findings will be discussed separately

The Effect of Age

Iron and copper concentrations, given as an average of the left and right lobes, are presented in Fig. 1 Direct inspection of the graphs suggests that there is no difference between the iron and copper concentrations with maturity. This was confirmed on statistical analysis. (The variance ratio (F) for iron being 1.5 for

TABLE 2 *Chemical results of the water content and the iron and copper chemical analyses based upon dry weight from the left and right livers of 75 apparently normal livers a d 11 children with haemolytic disease. The cases are listed in ascending maturity*

Weeks gestation	% Water L	% Water R	Iron L	Iron R	Copper L	Copper R
25	70	78	148	100	21 1	18.6
23	73	74	68	88	.9	8.6
23	80	81	78	83	16.3	16.8
26	83	82	189	148	17.0	17.0
26	80	80	140	115	16.1	16.4
26	79	79	46	41	15.2	12.3
26	76	77	100	96	11.7	13.5
26	78	77	82	40	10.9	13.3
26	80	80	170	100	11.6	8.5
26	80	79	103	81	23.1	1.8
26	78	77	88	52	10.3	7.9
28	81	80	100	90	14.2	12.9
28	77	75	135	96	16.9	14.7
28	78	78	95	82	16.4	18.0
28	81	82	126	128	10.0	10.3
29	79	78	119	118	20.9	16.6
29	78	79	80	39	13.[illegible]	1[illegible].9
29	82	81	106	100	15.9	15.8
29	81	80	188	145	1[illegible].5	11.3
29	82	81	278	232	15.7	13.4
30	78	78	30	29	34.5	32.0
30	77	6	187	188	16.7	17.1
30	77	75	204	204	20.8	20.9
30	85	83	59	39	—	—
30	82	81	172	121	13.9	11.3
30	84	84	119	106	12.6	12.6
33	81	80	48	190	22.8	17.9
33	78	77	177	165	12.7	14.1
3	78	79	204	162	19.0	19.0
33	81	80	105	85	14 4	12.1
33	82	82	47	40	14.9	18.0
33	80	79	80	81	20.2	14.9
33	77	77	130	112	21.5	23.0
34	88	90	43	48	8.1	4 8
34	76	76	71	54	16.4	15.7
34	81	84	68	61	17 1	14 7
34	83	83	152	189	8.3	7 6
34	87	86	79	66	4.0	26.2
34	86	85	83	48	6 0	8.8
34	78	77	166	167	24.4	24.2
35	79	79	17	172	1.8	19.3
35	77	77	167	165	[illegible]1	21.8
35	82	84	72	84	10.0	10.0
36	80	80	60	50	27.3	21.8

Weeks gestation	% Water L	% Water R	Iron L	Iron R	Copper L	Copper R
36	84	88	48	46	11.5	11.3
36	81	81	122	142	21.5	22.6
36	80	80	50	50	8.6	6.7
36	85	84	48	46	8.6	8.0
37	78	79	150	182	11.3	14.9
37	77	76	187	168	16.5	14.1
37	81	79	53	47	18.3	22.0
37	80	81	45	43	20 6	1.8
37	81	77	85	74	12.7	11.7
38	84	84	54	81	4.8	2.8
38	83	80	38	34	6.4	8.[illegible]
38	80	80	165	165	19 1	[illegible]
39	81	82	64	72	23.9	27.8
39	76	75	86	88	14.2	16.8
39	83	83	124	112	14.0	11.2
40	81	81	20	17	26.9	18.4
40	79	78	300	259	13.9	16.6
40	78	76	39	32	22.1	25.1
40	82	82	144	183	6.7	7.5
40	5	74	123	69	18.7	17.0
40	78	8	114	8[illegible]	8.4	6.1
40	79	80	204	145	1[illegible]1	14 6
40	77	77	157	157	8.3	10.8
40	76	75	248	253	20.6	23.3
42	78	78	127	77	13.7	11.3
42	78	75	49	38	11.1	9.3
43	80	79	170	101	12.5	15.0
43	84	83	43	55	13.7	11.3
43	77	77	117	109	18.0	19.7
44	80	79	106	119	11.8	13.2
44	84	82	61	69	18.8	22.6
Rh cases						
36	83	84	340	386	1[illegible]3	21.7
36	77	77	144	282	7 1	10.7
37	82	81	328	303	17.9	24.9
38	76	75	117	331	11.8	11.5
38	88	89	242	842	18.4	18.0
38	88	88	258	430	16.1	14 0
39	71	74	183	409	8.9	11.8
40	86	86	372	465	8.8	9.8
40	86	85	172	253	6.2	8.8
40	81	81	221	221	5.2	6.0
42	78	78	177	218	8 2	1[illegible]7

$V_1 = 70$ and $V_2 = 4$ $F(0.05) = 5.60$ how ing no significant difference between go groups.)

Similar lack of significant difference was found with the copper estimations. Thus there is no suggestion of any increase in iron or copper concentration with in creasing intrauterine maturity

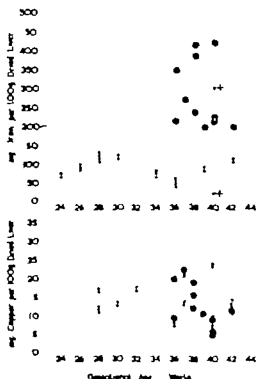


Fig. 1 The iron and copper concentration per hundred grams dried liver related to gestation age in weeks. The average of the two lobes of each case was taken for this chart. Children with haemolytic disease are represented with circles around the significant points.

Left and Right Lobes

The iron concentration of the left liver is higher than the right by an average difference of 9.43 ± 0.90 mg/100 g with a 95% certainty. The difference in iron concentration between the left and the right liver appears to be highly significant being significant at the 0.1% level.

Using a similar statistical technique of analysis it was found that there was no significant difference between the left and right lobes with regard to the copper concentration.

When the effects of age and the left and right lobes were tested for interaction effect, there was no interaction present. Therefore, it can be concluded that the age and the left and right values are independent for copper and iron.

Cases with Haemolytic Disease

The average iron and copper levels of the children with haemolytic disease are presented by large circles around dots in Fig. 1. The iron levels of the Rh cases are considerably higher than the normal range. In these cases, however, the right lobe is higher than the left lobe by an average difference of 10.00 ± 12.76 with a 95% certainty. This difference is significant at the 1% level.

When the left and right lobes of the Rh livers are compared with the normal series, there is a very significant difference. Regarding the right lobe, the mean for the Rh cases is 58.09 and for the normal cases 21.06, the standard error of difference being 5.20. Regarding the left lobe, however, the difference is not so great, the mean for the Rh cases being 30.00 and for the normal cases 14.32. The standard error of difference is 5.78. The difference, however, is significant at the 5% level only as distinct from the 0.1% level for the right lobes.

Regarding the copper concentration in the Rh cases there is no significant difference between the left and right lobes. There appears, however, to be a difference between age groups. The mean copper content for the Rh group 36 and under 40 weeks is 15.06 and for the group 40–44 weeks is 7.72, the standard error of difference being 2.48. On this evidence it appears that the difference between the age groups is significant at the 1% level.

Summary of Results

Two things emerge from this study concerning the normal series. First, that there is no increase in the normal copper and iron concentration of the liver during later stages of normal intrauterine lif Second that there is a significant difference between the iron concentration of the left and right livers in normal intrauterine life The iron concentration of the left lobe is higher than that of the right There is no difference between the copper concentration of the two physiological lobes.

In children with haemolytic disease there is a very great increase in the iron concentration in the liver This is present throughout both lobes but is more marked in the right lobe than in the left so that there is a complete reversion of the apparent physiological ratio of iron concentration. The copper concentration in these cases is significantly diminished only in the children surviving to full term.

Discussion

Our findings would suggest that the impression gained by some earlier workers that there is an increase in the iron concentration of the liver in later intrauterine life is probably due to the inclusion of some cases of haemolytic disease among their normal series. It is also interesting that in our own material we have found several cases in which the iron concentration was in the same region as those of children with known Rh disease and in whom no serological incompatibility had been found. This suggests t us that these children probably have suffered from some form of haemolytic disease that has not yet been discovered.

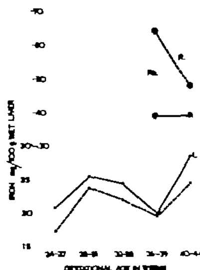


Fig. 2. The average iron concentration in the left and right liver of 78 "normal" livers and 11 livers from children with Rh disease. The latter are represented with circles around the significant points.

The peculiar difference found in the iron in the left and right lobes in the normals and the Rh livers came as a surprise to us (Fig 2) Under normal circumstances the left physiological lobe has less haemopoietic foci than the right and we have found that the iron content of the left side is higher than the right This is probably explained by the more rapid turnover of iron in the right than in the left liver The reverse ratio of concentration occurring in haemolytic disease is more difficult to explain. The amount of haemopoiesis seen in these cases varies very much. It would seem likely that, in the Rh cases we see secondary effects du to the child being in a stat of chroni relative anoxia. In such a state the left liver is in a favoured position as it has the most highly oxygenated blood, the right liver having perhaps the least oxy

genated blood in the body Thus, when toxic and anoxic changes are liable to occur it is the right liver that suffers rather than the left. Also the right liver is largely supplied with blood that does not go through the placenta and it seems possible that the placenta removes or fixes some of the pre-hepatic iron containing haemoglobin substances and thus shields the left liver from some of the effects of chronic haemolysis

Regarding the changes in the copper concentration seen in older children dying with haemolytic disease we are at a loss to explain this unless it could be that there has been a general depletion in copper reserves in the foetus due to the increased turnover of haemoglobin metabolism or perhaps an increased deposition in other organs, possibly the brain.

Summary

A study of the iron and copper concentration of livers from 75 apparently normal stillborns and 11 infants dying with haemolytic disease show that in normal circumstances there is no increase in the iron and copper concentration with increasing intrauterine maturity

There is no difference between the concentration of copper in the left and right lobes but there is a significant difference in the amount of iron the left containing more than the right

In children with haemolytic disease there is a gross increase in the iron concentration of the liver and this is more marked in the right than in the left lobe

In children with Rh disease there is possibly some diminution in the copper concentration of the liver in those cases that survive to full term

Acknowledgement

It is a pleasure to be able to acknowledge the assistance of Miss Doris Kirk of the Sheffield University Department of Statistics, and Dr A. J N Warrack, through whose courtesy many of the necropsies used in the study were available

References

1 Bateman, G and Zondek, S. G Iron, copper and manganese in human organs at various ages. *Biochem J* 33 1845–1857 1939.

2 Butt, E. M., Nusbaum, R. E., Gilmour, T C. and Didio, S. L.: Trace metal patterns in disease states. II. Copper storage diseases, with consideration of juvenile cirrhosis, Wilson disease and hepatic copper of the newborn. *Amer J Clin Path*, 30 479–497 1958.

3. Cartwright G E. Copper Metabolism, pp. 274–310 McElroy W D & Glass, B. (Eds.). Johns Hopkins Press, Baltimore 1950

4 Cunningham, I J Some biochemical and physiological aspects of copper in animal nutrition. *Biochem J* 25 1267 1294 1931

5. Eden A. and Green H H Micro-determination of copper in biological material. *Biochem J* 34 (?) 1202 1208 1940.

6. Emery J L.: The distribution of haemopoietic foci in the infantile human liver. *J Anat* 90 (2) 293–297 1956.

7 Gladstone, S. A.: Iron in the liver and in the spleen after destruction of blood and transfusions. *Amer J Dis Child*, 44 (1) 81 103, 1932.

8. Hugounenq, L. *Compt Rend Soc Biol* 51 337 1899 (cited by Yrenn xx).

9 Josephs, H and Sherman W W A study of fetal iron. *J Biol Chem*, 111 ?43 ?49, 1935.

10. Kaldor, I.: Storage iron in the foetus. *Aust J Med* 4 277 278, 1953.

11 Mall, F P A study of the structural unit of the liver *Amer J Anat*, 5 227–308, 1906.

12. MORRISON, D. B. and NASH, T. P. The copper content of infant livers. *J Biol Chem*, *88–89* 479–483, 1930.
13. NEEDHAM, J. Chemical Embryology 3. 1278, 1931 Cambridge University Press.
14. RAMAGE, H., SHELDON J. H. and SHELDON W. Spectographic investigation of the metallic content of the liver in childhood. *Proc Roy Soc* *113B* 308–327 1933.
15. RAMSAY W. N. M. and CAMPBELL, E. A.: Iron metabolism in the laying hen. *Biochem J* *48* 313–317 1934.
16. SCHWARTZ, BAER and WEISER: *Z Kinderheilk*, *37* 107 19 4 (cited by GLADSTONE).
17. SHELDON, J. H. and RAMAGE, H.: A spectographic analysis of human tissues. *Biochem J* *25* 1608–1627 1931.
18. SMITH, N J. ROSELLO, S., SAY M. B. and YEYA, K.: Iron storage in first five years of life. *Pediatrics*, *16* 166–173, 1955.
19. TOVERUD K. U. Investigation on the iron store of newborn infants. *Acta Paediat*, *17* (Suppl. No. 1), 136–139 1935.
20. WIDDOWSON E. M. and SPRAY C. M.: Chemical development in utero. *Arch Dis Child*, *26* 205–214, 1951.
1. ZALESKI *Z Phys Chem* *10* 453, 1886 (as cited by NEEDHAM).

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Chromosomal Abnormality in a Mongolism Like Syndrome[1]

by J. A. BÖÖK, K. H. GUSTAVSON and BERTA SANTESSON

The somatic chromosome number in man was unequivocally determined as 46 by Tjio & Levan (13) in 1956. Most patients with mongolism have 47 chromosomes (2, 7, 8). The extra chromosome[2] is morphologically identical with no. 21, i.e. it is a very small acrocentric chromosome with satellites. Preliminary data on meiosis in a few male patients indicate the occurrence of trivalents during the first metaphase (9). Consequently genuine trisomy is very likely.

This trisomic type of mongolism should be caused by the occasional production of gametes with 24 and 22 chromosomes through non-disjunction during meiosis. The gametes contain either two chromosomes no. 21 or none. The former fertilized by normal gametes give rise to children with mongolism. The fate of zygotes having 45 chromosomes, among which one chromosome no. 21 only, is unknown. This type of monosomy could be lethal at an early fetal stage. The well known association between mongolism and advanced maternal age indicates that non-disjunction is more common at oogenesis in women at the end of their reproductive period.

While trisomy for the 21st chromosome is the usual genetical explanation for mongolism, other cytological types have been found. It is not yet clear whether the clinical syndromes associated with these other chromosome abnormalities are identical.

Fraccaro *et al.* (5) found 46 chromosomes in a boy with mongolism. There were five chromosomes of group 19-20 instead of four and only one chromosome 21. The most likely explanation of this karyotype is that the new chromosome originated through centric fusion of two chromosomes no. 21.

Another type of translocation in a girl with mongolism was reported by Polani *et al.* (12). The chromosome number was 46 and the translocation interpreted as having occurred between chromosomes nos. 14 and 21.

The discovery of these translocations also explains the exceptional occurrence of several individuals with mongolism in the same sibship. Two such families have been reported from England (4, 11). In both cases the normal mother had a translocation between chromosomes 15 and 21 and a total of 45 chromosomes.

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[1] This investigation is part of a programme aided by grant from the Foundations' Fund for Research in Psychiatry, the Swedish Atomic Research Council, the International Atomic Energy Agency and the United States Atomic Energy Committee.

[2] The Denver nomenclature of human mitotic chromosomes has been used consistently in this paper (*Lancet* 1063–1065, 1960).

The affected children, having a total of 46 chromosomes apparently had inherited the translocation chromosome 15/ 1 plus one normal no. *1 from the mother and one normal no _1 from the father The common denominator for these non trisomic types of mongolism is that they should have nearly all the genetical code material of chromosome no _1 in triplicate The loss of chromosome material through the translocations is apparently very small.

We have observed a patient with a mongolism-like syndrome who displayed a new and different type of chromosomal aberration which was briefly mentioned in an earlier report (1) This patient is one of a series of 35 individuals with mongolism or suspected mongolism who have been karyotyped in our laboratory since 1950

Description of the P tient

S L is a girl born Oct 3, 1956. Her mother born in 1929, is healthy and S is her only child. There have not been any bortions. The father born in 19*3, is healthy and has three normal children by previous marriage N cases of mongolism, malformations, mental retardation or other significant diseases or defects are known among the near relatives. The mother was healthy during the pregnancy and received regular pre-natal care The patient was born at full term by an uncomplicated breech presentation Her weight at birth was 3320 g, her height 47 cm and her head circumference 34 cm. She suckled poorly but no other neonatal complications occurred.

The patient was admitted t a pediatric clinic in Stockholm at the age of 9 months because of constipation and retarded development. She had a slightl reduced muscular tonus and h perflexible joint Her height was 64 cm which is considerabl below the average. Her weight in relation to the height was normal. Her head circumference was 45 cm, which is normal for her age She had a broad nose bridge left-sided strabismus, smaller than normal palpebral fissures, bilateral epicanthus, a preauricular outgrowth on the left side and misshapen prominent ears. No physical signs of heart disorder were found.

Roentgenograms of the hip-joints showed both caput femori dislocated above the articular cavity (Fig. 1). Roentgenograms of the skeleton revealed an pproximat three months retardation in the development of the bone nuclei.

The serum cholesterol was 180 mg per 100 ml and the prot in bound iodine 7.9 µg per 100 ml.

An estimat of her development according t the Buhler Hetzer scale showed correspondence with a child of seven months, i.e. a retardation of two months.

Ophthalmoscopic examination t the eye clinic of Karolinska Sjukhuset in Stockholm revealed a retinal degeneration with scattered whit spots in both eye fundi. The optic discs were normal. An lectroretinogram showed no pathological hanges.

At the age of 15 months the patient was transferred to the orthopedic division of St Göran Hospital in Stockholm where the luxations were replaced and cast pplic l. On account of a continued tendency t dislocation, sub-trochanteric osteotomies w made on both femurs in January 1959 A further osteotomy was made on the left femur in October 1959 Since then she has had no dislocations.

Examinations at the Pediat ic Clini Karol nska sjukhuset in Stockhol

The patient was first admitted 1958 at the age of two year She readmitted several times for studies. The last time was

Her height has al y b b However her weight in r lat has been normal.

Roentgenograms of

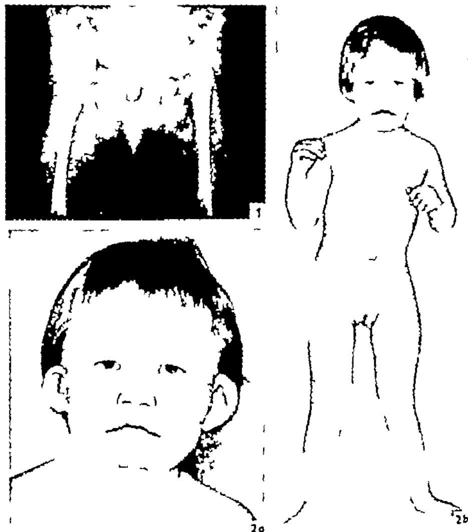


Fig. 1. Roentgenogram of the patient hip joints.

Fig. 2a and b. The 4 yea old girl with a mongolism like syndrome

somewhat small calvari m. No intracranial calcifications were visible. There were no deformities of the facial region or of the base of the skull. Roentgenograms of the skeleton showed a normal number of ossification centers with regard t her height but fewer than normal with regard t her age. The bones were slender. \ leform ties of the middle phalanges of he little fingers were visible. An intraveno urogram showed a normal anatomy. Roentgenograms of the heart and lungs were normal.

The electrocardiogram and the electroencephalogram were likewise normal. Physiological alues were obtained for hemoglobin and red blood cells. The whit blood cell count was 5000– 000 per cmm with a normal differential count. The fasting blood sugar was 109 mg per 100 ml, the serum cholesterol 210 mg per 100 ml, the serum

calcium 10.8 mg per 100 ml, the serum phosphorus 6.1 mg per 100 ml, the serum protein 7.1 g per 100 ml with normal lectrophoretic pattern and the protein-bound iodine 9 µg per 100 ml.

The Wassermann reaction was negative. A serological test of toxoplasmosis gave a negative result. Direct and indirect Coombs tests were negative. She had no proteinuria. The urinary sediment was normal. Screening of the urine for detection of metabolic disorders (after Lingen, 1960) comprising one and two-dimensional amino acid chromatography $FeCl_3$ reaction and dinitrophenyl hydrazin reaction revealed no pathological changes. N sugars indicating metabolic disease were found in the urine.

At 2 years of age the patient was clearly retarded in her development. Judged by fine motor movements her development was equivalent t child of 15 months, and with regard to gross movements to child of 11 months. Her social development was equl valent to child of about 12 months. A further estimat of her development made at the age of 3 years showed that the fine motor movements and the adaptive development were equivalent t that of a child of 2 years. The patient walked at the age of 2 years and 5 months. She could speak single words at the age of 3 years.

An estimate of her intellectual development at 3 years and 7 months of age showed general level corresponding t a 2½ year old child. The patient has always been easy t handle. She is cheerful and likes t sing and listen t music. Her general health has always been good. Apart from chronic blepharitis, no infections have been reported.

A physical examination in Aug 1960 (K. H. Gustavson) showed 3 years and 10 months old somewhat small girl with a rather odd ppearance (Fig 2) Her head circumference was 45 cm, the length 15.8 cm, the breadth 13.4 cm and the cephalic index 84 Her height was 88 cm and her weight 12 kg She had typical epicanthic eye fold on both sides. The palpebral fissures were narrow and she had slight blephantis bilaterally The nose was flat and relativel small with

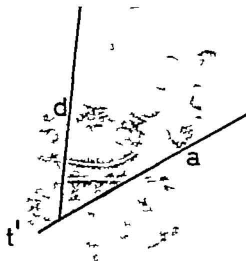


Fig. 3. Dermal pattern of the left hand of the patient. The axial triradius could be classified t' (maximal atd angle 53°).

broad bridge. The ears were misshapen and prominent. The tongue was of normal size and without sign of papillary hypertrophy or deep furrows, and not protruding. The position and the shape of her teeth were normal, but she had extensive caries and paradentosis. The hands were relatively short and broad with short little fingers. The feet were small and short and in typical plano-valgus position. The third toe was considerably lower set than the others. The joint were hyperflexible and she had a generally decreased muscular tonus. N physical signs of congenital heart disorder were found. The configuration of the thorax, the abdomen and the external genitalia were all normal. Further routine physical and neurological examination showed no pparent bnormalities. N four finger line was present.

The dermal ridge patterns of the hands and feet (cf. Fig. 3) were quit different from those usually found in mongolism. W are grateful to Professor L. S. Penrose who has discussed these details in the appendix.

Summary of Clinical Findings

The patient is a 4 year old girl displaying a moderate mental and physical retardation. She has certain symptoms and signs characteristic of the mongolism syndrome. However the dermal patterns of her hands and feet are not typical of this syndrome. She also has a bilateral congenital dislocation of the hips and signs of retinal degeneration.

The etiology is unknown. Thyroid disease is excluded and no evidence of an environmental cause has been disclosed. There are no signs of abnormal sexual development.

Cytological Observations

Chromosome studies were made according to the technique developed in this laboratory (3, 6). The cytological observations on the patient S.L. were based on 3–5 separate primary cell cultures and their subcultures from one bone marrow biopsy and two skin biopsies taken from the right and the left side of the body. The karyotyping of the parents was based on similar unilateral skin biopsies. The results have been summarized in Table 1.

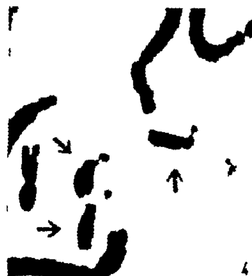


Fig. 4. Detail of a part of a metaphase plate. The arrows point to 3 satellited acrocentric chromosomes described in the text. Dermal cell culture of the patient.

The material derived from the patient gave sufficiently consistent results to exclude cytogenetical heterogeneity. Among 61 metaphases selected as being apparently undamaged 53 had exactly 46 chromosomes. The deviations shown in Table 1 are the usual ones to be accounted for by methodological errors. In all these 46-chromosome cells we found five short acrocentric chromosomes of group 21–22, i.e. one additional chromosome

Table 1. *Summary of chromosome analyses of the patient and her parents*

Individual and origin of cultured cell	Chromosome counts 43	46	46 ± 3	47	Total number of analysed cells	Chromosome complement
S. L. bone marrow	1	13	4	1	19	Trisomic 21, monosomic 15, or translocation 15/21
S. L. skin		4			42	
Mother skin		18	1		40	Normal
Father skin	1	19	1		20	Normal

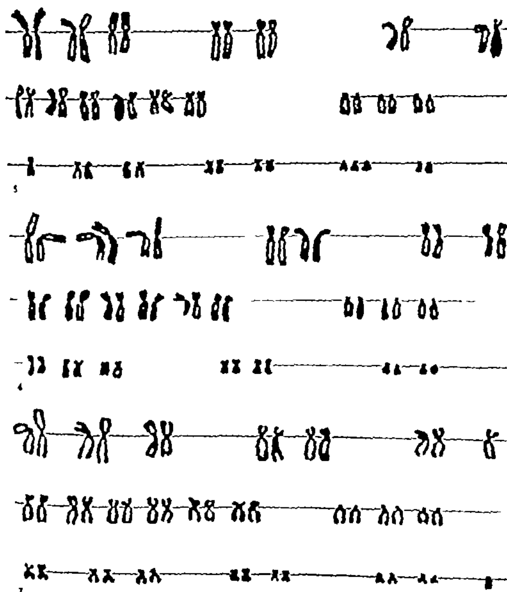


Fig. 5 Chromosom[illegible] ple [illegible] f [illegible] pa[illegible] interpreted as monosomic 16, trisomic [illegible] 1 or as [illegible]nsh[illegible] in [illegible] the short arms of 16/21

Fig. [illegible] Ap [illegible] al [illegible] mosome complement of the mother

Fig. [illegible] Ap [illegible] normal [illegible] mosome complement of the father

Eighteen cells derived from all three biopsies were subjected to a detailed analysis with the aid of enlarged microphotographs. In four of these three small acrocentric chromosomes with satellites could be seen clearly (cf. Fig. 4). By morphological criteria these should be 21 s. Ordinarily group 16–18 has six chromosomes, but in this case only five were recovered. By arranging the chromosomes of this group according to arm length and centromere position the odd chromosome was considered to be no. 16. The karyotype has been exemplified in Fig. 5. Typical sex chromatin was found in 50–60 % of the interphase nuclei of skin and oral mucosa cells.

The cells derived from the parents showed the normal diploid number of chromosomes. The chromosomes of four metaphases from each parent have been matched and analysed in detail. The normal karyotypes are exemplified in Figs. 6 and 7.

The reliability of the diagnosis of individual karyotypes by the techniques used in this laboratory has been discussed in previous papers (3, 6).

While several interpretations of the karyotype of this patient are theoretically possible, the following two alternatives appear most likely:

(1) The additional chromosome in group 21–22 is a third chromosome no. 21. The patient thus should be trisomic 21 and monosomic 16. This postulates a process of non-disjunction during meiosis in one or both parents. The zygote should be the result of a combination of a normal haploid gamete and one gamete lacking chromosome no. 16 and at the same time having two 21 s, or a combination of two gametes one of which had the haploid set plus an additional chromosome no. 21 and the other one having the haploid set minus one chromosome no. 16.

(2) The additional small acrocentric chromosome originated during gametogenesis in either parent by a translocation between nos. 16 and 21. It should consist of the small arms plus the satellites of no. 21 and the short arms of no. 16 assuming a point of exchange close to the centromeres.

At the present time we cannot give a preference to either of these two interpretations. The remarkable fact remains that this patient lacks a considerable amount of chromosome material even if definite monosomy is doubtful. The situation is, however, not unique as this patient from a cytogenetical viewpoint is very similar to another patient with a congenital heart defect who was investigated in this laboratory (3). The alternatives in that case were a combination of trisomy for 19–20 and monosomy for 22 or a 22/19–20 translocation.

Comments

The diagnosis of mongolism is usually based on the presence of mental deficiency in combination with three or more of the following signs: (1) a brachycephalic skull, (2) oblique palpebral fissures, (3) epicanthic fold over either eye, (4) fissured tongue, (5) blepharitis, (6) transverse fold on either palm, (7) short crooked fifth finger. There is, however, no general agreement as to which combination gives a safe diagnosis. With the discovery of the specific genetical etiology of this syndrome such arguments have become pointless. The question whether or not the term mongolism

should be kept to signify a special clinical syndrome will depend on which differences, mental, physical or biochemical, will be found between patients with different karyotypes. So far it is clear that mongolism does not constitute a clinical and genetical entity.

On the clinical signs and symptoms the patient reported here could be grouped as a case of mongolism. Nevertheless, in as much as this patient is genetically entirely different from the common trisomic type as well as different from the translocation types so far reported, we are concerned with a new genetical disease entity. From this point of view one might argue that the patient also represents a new clinical syndrome.

Points of particular interest are the occurrence simultaneously with a number of signs often connected with the mongolism syndrome, of congenital dislocation of the hips and retinal pathology. Neither of these two changes have significant associations with mongolism (10). Their significance in this patient will remain obscure until further cases with the same karyotype have been found. The presence of these two defects may be accidental or due to the expression of recessive genes located in monosomic chromosome segments. In both instances these defects would not form consistent parts of a clinical syndrome.

Summary

The cytogenetical and clinical observations of a 4 year old girl with a mongolism-like syndrome are reported. The patient showed a moderate mental and physical retardation. Some signs and symptoms considered characteristic of mongolism were present. Others, such as the dermal patterns of hands and feet, rather contradict this diagnosis. In addition the patient had a bilateral dislocation of the hips and unspecific signs of retinal degeneration. There were no signs of abnormal sexual development.

Chromosome studies based on cell cultures derived from bone marrow and skin biopsies revealed a chromosome number of 46 and a consistent karyotype pattern which was interpreted as a combination of trisomy for chromosome no. 21 and monosomy for no. 16, or alternatively as a translocation between the short arms of 16/21. Typical sex chromatin was present in 50–60 % of the interphase nuclei. Both parents had apparently normal karyotypes and were free from signs or symptoms of significant disease.

This patient represents a new genetical disease entity and clinically a variant of the common trisomic type of mongolism.

References

1. Böök, J. A.: Mongolism and other conditions associated with chromosomal aberrations. *Acta Soc. Med. Upsal.* 65: 1–2, 1960.
2. Böök, J. A., Fraccaro, M. and Lindsten, J.: Cytogenetical observations in mongolism. *Acta Paediat. (Uppsala)* 48: 453–468, 1959.
3. Böök, J. A., Santesson, B. and Zetterqvist, P.: Association between congenital heart malformation and chromosomal variations. *Acta Paediat. (Uppsala)* 50: 217, 1961.
4. Carter, C. O., Hamerton, J. L., Polani, P. E., Gunalp, A. and Weller, S. D. V.: Chromosome translocation as a cause of familial mongolism. *Lancet* I: 678–680, 1960.

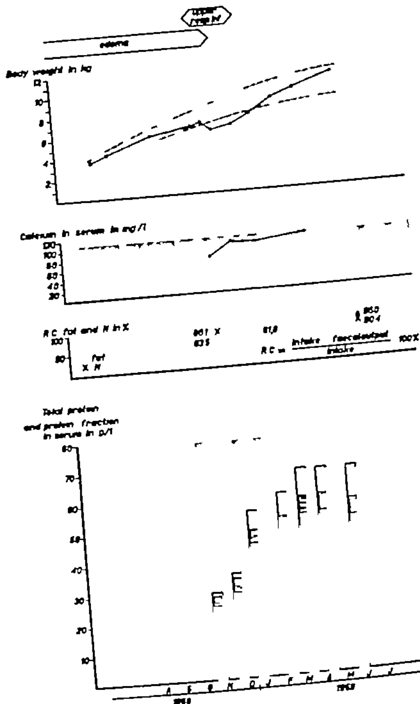

upper resp.inf
edema
Body weight in kg
Calcium in serum in mg/l
R C fat and N in %
R C = intake - faecal output / intake 100%
Total protein and protein fraction in serum in g/l
1960
1962

Fig. 1

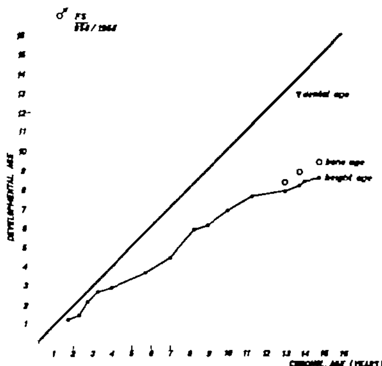


Fig. 2.

No loss of proteins with the stools can be demonstrated and nitrogen resorption will be normal. Gordon demonstrated this leakage by the intravenous injection of radioactive labeled polyvinylpyrrolidone (PVP); this compound, which has an average particle size somewhat lower than that of serum albumin, cannot be metabolized after leakage into the intestines.

In 9 patient with essential hypoproteinaemia, Gordon found much larger part of the injected PVP in the stools than in 41 normal controls. He suggested the name exudative enteropathy for these cases of hypoproteinaemia (19).

Since Gordon first experiments, other cases of essential hypoproteinaemia have been published in which also an increased loss of injected labeled PVP with the stools was reported.

Case Histories

Patient A., a female infant, developed hypoproteinaemia at the age of four months. Serum protein values: 30 g/L total protein, of which albumin 59 9%, -globulin 7.3%, a_2-globulin 17 4%, β-globulin 13.1%, γ-globulin 3.3%. Serum calcium was 77 mg/L, intake and resorption of protein was normal. There was no albuminuria but there was moderate hyperaminoaciduria with a normal pattern. Fat resorption coefficient was only 64% both on glutenfree and normal diet. Liver pancreatic and renal functions were normal. \ PVP test was performed in this patient.

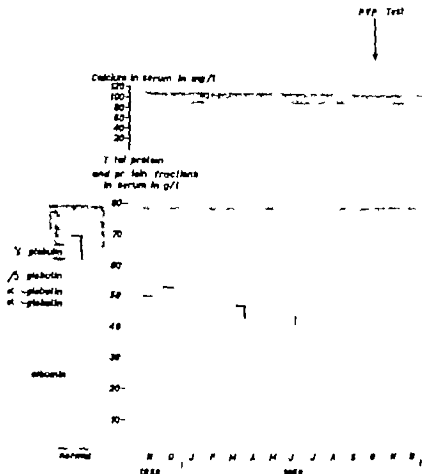


Fig. 3.

The diagnosis was essential hypoproteinaemia. Figure 1 shows the course of the disease. The child recovered spontaneously; the return to normal of serum proteins was accompanied by an increase of fat resorption to normal values, indicating a relationship between the two phenomena. Aminoaciduria also became normal. She is an example of the transient infantile form of essential hypoproteinaemia (4, 1?).

Patient B., a boy of 13 years of age, was investigated because of stunted growth with mental deficiency (IQ 63), for which no specific cause could be found (Fig. 2). Hypoproteinaemia (49 g/L) (Fig. 3) was an incidental finding since there were no clinical symptoms. Thorough investigation did not disclose a cause for the low serum proteins. Serum calcium, fat resorption and aminoaciduria were normal. A diagnosis of essential hypoproteinaemia was made.

The boy suffered from stomach-cramps; a year before a duodenal ulcer was found (Dr. Huysinga) and at the time of our study there was X-ray evidence of duodenitis and jejunitis (Fig. 4). A PVP test revealed an excessive loss of PVP with the stools.

Patient C., a girl of 14 years of age, had generalized oedema since birth (Fig. 5). There was also chronic diarrhoea. Mental and somatic development were retarded (Fig. 6). The main biochemical data are summarized in Fig. 7. As no cause for the hypoproteinaemia was found, the diagnosis

Fig. 4. Roentgenological findings in patient B.—A. The duodenum descendens has lost its mucosal pattern. The contours are coarse, and the duodenum is partly widened. B. The jejunum has also a coarse mucosal pattern and contains rather much secretion. Segmentation and scattering of the barium contrast is noted.

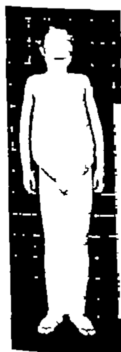


Fig. 5.

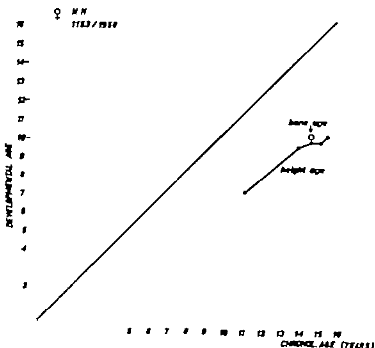


Fig. 6.

TABLE _ Compos[illegible]

Date	9 9 1958	1 10 1958	13.10 1958	21.10 1[illegible]
Diet			81 g fat day	81 g fat [illegible]
Volume in ml	11,200	7000	100	400
Colour	milky	milky	milky	milky
Total protein in g/l	20			18.3
Total protein loss in g	224			[illegible]
T[illegible]tal lipoid in mg/100 ml	500		513	420
Cholesterol in mg/100 ml	78		61	65

patients B and C than in the control subject The same phenomenon was found in the study of Schwartz (30) From the limited number of observations it is difficult to conclude that this is a real difference The result is possibly due to a higher renal clearance of I^{131}–PVP during the first day of the test caused by a higher average blood level of this substance in the control than in the patients B and C. (Compare also $t_{\frac{1}{2}}$–values in Table 3) However several other hypotheses are possible

Young (47) demonstrated that 77 % of the injected I^{131}–PVP was retained in the body in his patient after 3 days, mostly in the liver which quantity further decreased by about 1 % a day

Fig 8 shows the results of all PVP tests published up to November 1930 The data for our patients lie well outside the normal range A more extensive and technical description of the PVP test in our patients had been published elsewhere (45).

Since the outlook for patient C was poor and both steatorrhoea and the PVP-test results suggested intestinal abnormalities or disease it was decided to perform a laparotomy It has been shown (1 39) that in some cases of essential hypoproteinaemia surgical intervention can lead to cure. The operation was performed on

TABLE 3 *The cumulative excretion (E) in urine (U) and faeces (F) and the relation (100 minus E) of I^{131} PVP calculated in percents of injected dose respectively after 4 and 7 days after injection The disappearance rate of I^{131} PVP from the plasma is given as* $t_{\frac{1}{2}}$

	Idiopathic hypoproteinaemia						Oligophrenia		
	Patient B			Patient C			Control		
Time after injection of I^{131} PVP	Excretion U	Excretion F	Retention	Excretion U	Excretion F	Retention	Excretion U	Excretion F	Retention
4 days	1[illegible].0	4.0	84.0	14.1	4.0	81.9	28.7	1[illegible]	70.1
[illegible] days	13.7	4.3	82.0	15.5	4.5	80.0	20.3	1.8	67.9
$t_{\frac{1}{2}}$ between 0 and 24 hours after injection	5.[illegible] Hours			8.7 Hours			13.1 Hours		

ies 1 patient C

10 1959	16.9 1959	2.11 1959	7 1 1960 Laparotomy	24.3 1960	11.4 1960 Laparotomy
g fat/day		85 g f t/day		53 g fat/day	63 g fat/day
3000	11 000	8400	5800	6700	± 6000 cc
wish turbid	milky	milky	milky	yellowish turbid	yellowish turbid
10 5		17	19	21	18
49.5		14_8	110.2	207 7	± 108
209		264	232	104	
62		74	94	87	

January 7 1960 The small intestine showed a much thickened wall with increased vascularity and a purplish red colour In the distal 100 cm these signs were less pronounced. The omentum was reduced to a course mesh and there was no omental bursa (Fig 9) The stomach, duodenum, and large intestine had a normal appearance

Biopsies of the small intestine at several levels revealed remarkable microscopic changes.

The mucosa was swollen and chronicly infiltrated The submucosa was oedematous, showing large histiocytes with foamy protoplasm (Fig 10) The number of these macrophages increased in distal direction and reached a maximum in the second half of the jejunum and the beginning of the ileum. In the muscular layer a yellow brown pigment was seen, increasing in quantity in the distal direction. The greatest concentration of the pigment was found in th distal part of the ileum The

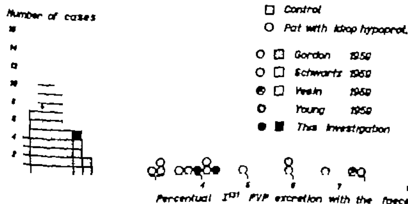


Fig. 8. Frequency-distribution of he percen ual PVP-excretion with the faeces in 52 controls and 17 patients with idiopathic hypoproteinaemia.



Fig. 9.

mesenteric lymphnodes also contained large quantities of this pigment. Extensive histochemical investigation showed that these macrophages contained protein like substances and no fat. The pigment could be classified as a lipofuscin.

The biopsies of stomach, large intestine and liver showed no abnormalities except oedema.

The most probable explanation for these pathological findings seemed to us to lie in a direct relation hip between the large cells in the submucosa and the loss of protein. It was assumed that these cells might take up proteins leaking from the vessels into the intestinal lumen. The pigment was con idered to be a secondary phenomenon. It has been described in other cases of hypoproteinaemia (35) but in these cases it w not confined to the small intestine. If this theory was correct the main "protein leaking" part of the small intestine would be the part where most macrophages were found. We therefore decided to perform a resection of this part.

The patient recovered without complication from the first operation. On April 11 1960 a second laparotomy was performed. Twenty four hours and again one hour before operation small doses of human serum albumin labeled with fluorescein were injected intravenously. During the operation biopsy samples were again taken from the small intestine at several levels. Frozen tissue slices were prepared and studied both with a fluorescence-microscope and after haematoxylin-eosin staining. The findings were the same as at the first operation. The fluorescein-labeled

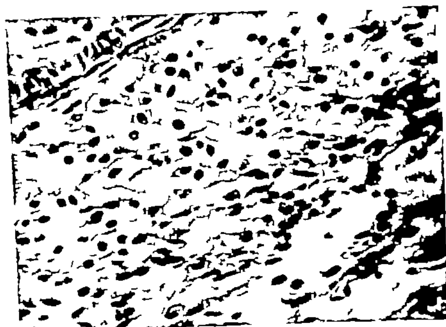


Fig. 10.

albumin was found in large quantities in the submucosal macrophages.[1] The part of the small intestine with the largest number of these pathological cells, according to both microscopical methods, was resected, 30 cm distally from the ligament of Treitz up to 230 cm from the ligament of Treitz.

The patient's recovery from the operation was quick and uneventful. During a short period after both operations a slight increase in total serum protein was noted. As Figure 7 shows, this effect is mainly due to a rise in plasma globulins, especially gamma globulin and not of plasma albumin. One month after both operations, the serum protein level was as low as before surgery. The resection has not cured our patient. In all probability the remaining intestine is too severely diseased.

Discussion

Our first patient showed a close relationship between hypoproteinaemia and steatorrhoea. Nevertheless, pancreatic function, gluten-tolerance and nitrogen resorption were normal, so that steatorrhoea was not the cause of the hypoproteinaemia. This suggests an intestinal lesion causing both symptoms. The second patient had hypoproteinaemia, normal nitrogen resorption, roentgenological duodenitis and jejunitis, and a pathological PVP test. The third patient showed all the clinical symptoms of essential hypoproteinaemia

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[1] We thank Dr R. O d. Heal and Dr J. Oerdt, of the Department of Pathology, University Hospital, Leyden, for their histological and histochemical examination and for suggesting and carrying out the method of fluorescein labeling of human serum albumin.

with steatorrhoea, a pathological PVP test and distinct macroscopical and microscopical lesions of the small intestine at laparotomy

Taken together the findings in these three patients are a strong argument for the theory that many patients with an unexplained hypoproteinaemia are in fact suffering from an exudative enteropathy (Gordon) In some cases, this diagnosis may lead to surgical intervention and cure (21 30)

It is possible that in many normal individuals a small amount of serum protein leaks into the intestinal lumen in patients with essential hypoproteinaemia this leakage exceeds the normal limits (21) The most important factor in the hypoproteinaemia in these cases is the hypoalbuminaemia, probably because of the small molecular size of plasma albumin as compared with plasma globulins The hypocalcaemia can be explained by the decrease of the protein bound fraction of serum calcium (23 29 41)

The exact nature and mechanism of protein loss into the diseased intestine is not yet known. Pathological and histochemical studies of the biopsies after injection of fluorescein labeled albumin may teach us more

Summary

Three patients with essential hypoproteinaemia are described. In all of them, signs and symptoms suggested an intestinal lesion. In two of them a PVP test was performed and gave a pathological result. Our cases and those of others strongly support the hypothesis that many cases of unexplained hypoproteinaemia are caused by intestinal disease The poor general condition of one of the patients led us to perform a laporotomy Biopsies showed distinct lesions of the small intestine A large part of the diseased small intestine was resected without benefit.

In some chronic cases surgical intervention may be tried The PVP test as first described by Gordon, is of great diagnostic aid in such cases.

Acknowledgement

We wish to thank Dr R S Gordon, Jr for his generous provision of I131-PVP

References

1 Albright F Bartter, F C. and Forbes, A. P The fate of human serum albumin administered intravenously t a patient with idiopathic hypoalbuminaemia and hypoglobulinemia. *Trans Ass Amer Physns 62* 204 194..
2. Bendich A. and Kabat E. A.: Immunochemical estimation of the rate of disappearance of transfused gammaglobulin from the blood in two cases of hypoproteinemia *J Lab Cl Med 34* 1066, 1949
3. Brooks, M W and Kyser, N M.: General edema of undeterminat etiology *JAMA 109* 1 193
4. Bound J P and Hackett W R.: Idiopathic hypoproteinaemic oedema and amino-aciduria in an infant *Arch Dis Chil. 28* 104 1953.
5. Cattell, W R. and Ahrens, T Idiopathic hypercatabolic hypoproteinaemia. *Proc Roy Soc Med 50* 314 1957
6. Citrin Y Sterling K. and Halsted, J.: The mechanism of hypoproteinemia associated with giant hypertrophy of the gastric mucosa. *New Engl J Med, 25* 906, 1937
7 Cornelis, L., Malbrai H and Vrancken, M.: L'hypoprotéinémie essentielle chez l'enfant. *Acta Paediat Belg 4* 337 194.

8 Crepai, K.: Über isoliert Störung des Salzstoffwechsels bei einem Fall on polyglandulärer Sklerose. *Kli Wsch* 2 1069, 1923.

9 Debré, R., Royer, P Lestradet H. and Rekeb, D L'hypoprotéinémie idiopathique de l enfant. *Sem Hôp Paris* 29 1909 1953.

10 Denham T J Idiopathic hypoprotein emia. *J Pediatrics* 41 440, 1937

11. Delaitre, Forty U and d'hypoprotéinémie d'apparence essentielle vec hypogam maglobulinémie chez un enfant de deux ans. *Arch Franç Pédiat, 11* 641 1954

12. Dvorak, J Kubovy A., Zavyorka, Z. and Hora, J Ein Fall von idiopathischer Hypoalbuminämie mit Aminoacidurie beim Säugling. *Helv Paediat Acta*, 9 258, 1950.

13. Ehrenbout W Die angeborene idiopathische Ödemkrankheit mit Hypoproteinämie. *Z Kinderheilk*, 74 141 1954.

14. Walter Falk, W and Larow, E.: Idiopathic hypoproteinemia. *Ann Paediat (Basel) 184* 80, 1955.

15. F ucoli, G.: Über Störungen der Wasser Kaushalts und Plasmaeiweißhaushalten. *Schweiz Med Wschr 78* 791 1948.

16 Fowler, J F and Miles, J M.: Rapid protein breakdown in idiopathic hypoproteinaemia. *Strahlentherapie*, Sonder band 38, p. 298, 1958.

17. Freeman T and Matthews, C M. E.: Analysis of the behaviour of I[131] albumin in the normal subject and nephrotic patient. *Strahlentherapie* Sonderband 38, p. 283, 1958

18. Gordon, R. S Jr: The preparation of radioactive polyvinyl pyrrolidone for medical use. *J Polym Sci, 31* 191 1958.

19. Gordon R. S. Jr: Exudative enteropathy *The Lancet, I* 325, 1959.

20. Hecht-Johansen A Hypoproteinaemia. *Acta Path Microbiol Scand*, Suppl. 33 p. 272, 1938.

21 Holman H a. Hypoproteinemia antedating intestinal lesions and possibly due to excessive serum protein loss into the in testine *Amer J Med 27* 963, 1959

22. Holten C.: Et tilfaelde af hypoproteinaemia. *Nord Med*, 11 2039, 1941

23. Hopkins, T R., C nvon, T B and Howard J E.: Ultrafiltration studies on calcium and phosphorus *Bull Johns Hopkins Hosp*, 91 249 1952

24. Illingworth, R and Finch E. Chronic generalized oedema and hypoprotein aemia of obscure origin *Arch Dis Child*, 29 813, 1954

25. Iverson, T (Idiopathic hypoprotein aemia. *Acta Paediat 47* 7?? 1958

26. J ????, E. Essentiele hypoproteinaemie Thesis, U trecht 1937

27 Johnson B J and Lagerkrantz, V Severe hypoproteinemia probably caused by hepatic damage *Acta Paediat 39* 185, 1950.

28. Kinsell, L W and Margen S. e.a.: Studies in methionine metabolism III. The fat of intravenously administered S[35]-labelled methionine in normal adult males, in patients with chronic hepatic disease, idiopathic hypoproteinemia and Cushing syndrome *J Clin Invest, 29* 238, 1950.

29 McLean F C. and Hastings, A. B.: Clinical estimation and significance of calcium-ion concentrations in the blood. *Amer J Med Sci, 189* 601 1935.

30 Mitchell, H H.: Adult growth in man and its nutrient requirements. *Arch Biochem, 21* 335, 1949

31. Meyer-Bisch R.: Über isolierte Störungen des intermediären Salzstoffwechsels und ihre klinische Bedeutung *Klin Wschr 4* 588, 1925.

32. Murato-F ??, J C., Castiglioni, C. and Amido, J B Oedème mous, hypoprotéinémie, et anachlorhydrie chez une malade atteinte de goitre exophthalmique. *Rev Neurol (Paris) 41* 758, 1934.

33. Nonnenbruch, W and Kudlich, H Chronischer Ödemzustand vom nephrotischem Typus mit Achropaxie bei chronischer Gastro-enteritis. *Med Klin*, 841, 1931

34. O'Donovan D K. McGrath, J and Galvin C.: Hepatic disease with chronic oedema as sole linical feature. *Irish J Med Sci*, 6 519 1950

35. Pappenheimer, A M. and Victor, J Ceroid pigment in human tissues. *Amer J Path, 22* 395, 1946.

36. Pearson J D Veall, N and Vetter, H.: A practical method for plasma albumin turnover studies. *Strahlentherapie*, Sonderband 38, p. 290 1958.

37 Salomonsen L Oedema causae ignotae *Nord Med 25* 727 1945.

38. Schönenberg, H Essentielle Hypoproteinaemie mit Tetanie. *Ann Paediat (Basel) 183* 333 1954.

39. Schwartz, M and J ???, S. Gastrointestinal protein loss in idiopathic (hypercatabolic) hypoproteinaemia. *The Lancet I* 237 1959

40 Schwartz, M and Thom ?? B Idiopathic or hypercatabolic hypoproteinaemia. *Brit Med J I* 14 19 7

41 Terepka, A. R.: The ultrafiltrable calcium of human serum II. Variations in disease states and under experimental conditions. *J Clin Invest 37* 87 1958.

42 Thom G W and Renold A.E.: Pathophysiology of edema. *Helv Med Acta 21* 331 1954.

43. ULSTROM, R. A., SMITH, N. J. and HEIMLICH, E. M.: Transient dysproteinemia in infants. I. Amer. J. Dis. Child, 92: 219, 1956.
44. ULSTROM, R. A., NAKAMURA, K. and HEIMLICH, E. M.: Transient dysproteinemia in infants. II. Studies of protein metabolism using amino-acid isotopes. Amer. J. Dis. Child, 93: 536, 1957.
45. VINK, C. L. J., DOOREN, L. J. and VAN GELDEREN, H. H.: to be published.
46. VESIN, P., RENAULT, H. and CATTAN, R.: L'hypoprotéinémie idiopathique. Étude de son mécanisme par le test au polyvinyl-pyrrolidone—I[131]. Bull. Soc. Méd. Hôp. Paris, 75: 319, 1959.
47. YOUNG, W. F. and LEVIN, B.: Gastrointestinal protein loss in severe mal-absorption complicated by idiopathic (hypercatabolic) hypoproteinaemia. The Lancet, II: 85, 1959.

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The Morphological Development of Pulmonary Arteries During the First Years of Life

by H. HERZENBERG and V ESKELUND

Over the last few years, there has been an increasing interest in the pathology of the pulmonary vessels in congenital and acquired heart disease. The changes in the pulmonary vessels have been shown to play an important part in the symptomatology and prognosis of these diseases. In the last decade, great advances in cardiac diagnosis and surgery have made surgical intervention possible, even during the first years of life It is very important that clinical data and laboratory findings be correlated with changes in the pulmonary arteries in congenital heart disease, especially during this period of life. In this way the indications for operation can be more definitely established.

In order to evaluat changes in the pulmonary vessels accurately an understanding of the normal conditions is a prerequisite. The following is a report on the results of a morphological study concerning the development of pulmonary arteries in children during the first years of life who did not have respiratory or circulatory diseases

Reports on the development of pulmonary arteries during the first years of lif can be found in the literature, but these are relatively few and the methods vary For this reason, a direct comparison is difficult. Brenner's nomenclature, from his classic work on the pathology of the vessels of the pulmonary circulation (2) has, as a rule been the basis for these reports. He divides the intrapulmonary branches f pulmonary arteries into three types: elastic arteries, muscular arteries and arterioles. According t Brenner definition, the *elastic arteries* are the largest, with a diameter of 1 mm or more, and they are accompanied by cartilaginous bronchi. They have a thin intima which lies directly on thick, elastic lamina interna. The media is composed of concentric lastic membranes between which are found bands f smooth muscle The adventitia is made up of rather coarse fibrillar collagen interwoven with fine elastic fibrils. There is no sharp delineation between the lastic arteries and the *muscular arteries* which ha e an outer diameter varying between 0 1 mm to bout 1 mm. These accompany the bronchioli. The thin intima rests on an internal elastic lamina, and the media is composed of circularly arranged smooth muscle between the internal and external lamina. In all of the larger muscular arteries fine elastic fibrils are present in the media. The adventitia resembles that of the elastic arteries.

The muscular arteries merge into the *arterioli*, which are defined as vessels with a diameter of 100 μ or less. According t

Brenner the vessel wall is composed of an endothelial tube surrounded by a single elastic membrane which is sometimes interspersed with individual cells of smooth muscle. No elastic externa has been demonstrated by Brenner. It should be pointed out that Brenner classification is concerned with the conditions found in adults.

Civin & Edwards (3) have shown that the intrapulmonary arteries and arterioles undergo a gradual development from fetal life until adulthood. At birth, the smaller pulmonary arteries resemble corresponding vessels of the systemic circulation. The lumen is narrow and the walls thick. These undergo fundamental changes during the first six months of life. The lumen becomes wider and the vessel wall thinner. Civin & Edwards work has been verified by Dammann & Ferencz (4, 5), Rosen, Bowden & Uchida (11) as well as Valsonzuela, Toriello & Thomas (13). In a special study concerning the development of fetal pulmonary arterioli, O'Neal, Ahlvin, Bauer & Thomas (9) have shown that the amount of smooth musculature in the vessel wall gradually increases during the last part of intrauterine life. They reported that the internal elastic lamina could be demonstrated as early as the latter part of fetal life even though it was thinner than the external elastic lamina. These authors were thus able to show two elastic membranes in contrast with Brenner findings, although his material included only adults but no infants or children.

Materials and Method

Lung preparations were obtained from autopsy material of 66 patients who died of diseases not primarily affecting the respiratory or circulatory organs. A paraffin block of formalin fixed lung was sectioned (most often several from each patient and from many lobes) and the sections were stained according to a modification of Gomori' elastin staining developed by V. Eskelund. According to this method the elastin was stained with formalin-fuchsin, after which the counterstaining (Van Gieson) was done. In this way the elastic tissue was stained dark blue-violet and was very distinct.

The vessels are classified according to Brenner's schema, but with one modification. The term "*elastomuscular arterie*" is used when the vessel resembled an intermediate stage between elastic and muscular arteries. These vessels have 2–3 concentric elastic bands in an otherwise muscular media. In addition, the internal and external laminae are relatively thick (Fig. 4). The muscular arteries according to Brenner are divided into *larger muscular arteries* (thus vessels lying near the peripheral branches of the bronchial tree where respiratory columnar epithelium could be still distinguished) and into smaller muscular arteries. The latter are *precapillary muscular arteries*, which either directly or with arterioli as intermediaries, branch into capillaries. This grouping is made because of the impression that larger and smaller muscular arteries are not always affected by pathological process to the same extent. Histologically the precapillary muscular arteries can be seen to branch from a larger muscular artery lying near a respiratory bronchiole (thus one with respiratory columnar epithelium). Other precapillary muscular arteries may lie surrounded by alveoli and at a distance from the bronchioli. The precapillary arteries resemble the muscular arteries, and therefore have a definable muscle layer and an internal and external elastica (Fig. 12). Only under pathological conditions is an elastic tissue found in the muscle layer of the media. During the first months of life the diameter of the lumen for these vessels can be as little as 10 µ. Arterioli of the type described by Brenner have not been subjected to determination in this material. Various authors define these vessels differently, as it is very difficult to distinguish between arterioli and venules.

The following measurements were made: *diameter of the lumen, wall thickness* (consisting of intima and media including external and internal elastic lamina), and *width of adventitia*.

Measurements were taken using a scaled ocular which was adjusted to the user and microscope. As far as possible vessels with circular lumen were measured (thus vessels hich had been cross sectioned). In those cases where such were not present the smallest diameter of oval-shaped lumens as calculated. In bliquely sectioned vessels, the thinnest width of the wall was measured in order t avoid what might simulate a medial hypertrophy. W th regard to the adventitia, which even in cross-sectioned vessels varies in width, an a erage value as obtained from measurements of the widest and thinnest parts. The values for the adventitia are therefore pproximate. At least 10 vessels f larger as well as precapillary muscular artery type were measured. It was not always possible how ever to find so many lastic and elasto-muscular arteries in the preparations. On the basis of these measurements, verage values were obtained and the variations were recorded. The following ratio was calcu lated: *Average lumen/average wall thickness (L/M)*

The different ratios at individual ages were graphically compared. Certain cases f chronic diseases and retarded development where the primary disease did not have its origin from changes in the respiratory and circulatory organs, howed pronounced diver gence in the figures, and were therefore not included in the study. It ought to be pointed out that this material, which was fixed in formalin, only partially reflects the conditions ivo, where the vessel calibre greatly aries according to different phy siological conditions.

Results

Each of the four different vessel types has been described according to morphological age development.

Elastic arteries. The size of the lumen at birth and some days afterwards is on the a erage about 300–400 μ which



Fig. 1. Elastic pulmonary artery of an infant 2 days old. The internal elastic lamina is thick and prominent and there are numerous elastic fibrils in the media. Part of bronchial cartilage in lower left field. Ratio lumen wall thickness low (4.0). (This as well as following preparations stained with formalin-fucsin and counterstained with Van Gieson connective tissue stain; 100.)

corresponds to about one third that found in adults. The internal elastic lamina is thick and wavy and the same is true of the elastic fibrils which are found in the media (Fig. 1). These contain quite a few smooth muscle cells. Only in certain exceptional cases can collagen fibrils be seen in the outer edge of the media. The external elastic lamina is not quite as thick as the internal, and is sometimes divided. The adventitia is made up of tightly arranged, rather coarse collagen fibrils. Between these are found fine elastic fibrils extending as far as the outer edge but seen mostly near the external elastic lamina. The adventitial layer is as a rule considerably wider than the media. The ratio between the lumen and wall thickness (L/M) 1 5– (Fig. 2).

By –3 weeks of age the size of the lumen has increased while the wall thick ness is to a large extent unchanged. The ratio L/M is therefore larger than earlier

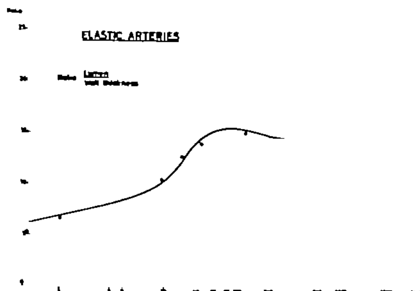


Fig. _. Elastic arteries. Ratio lumen wall thickness.

(7–14) The adventitial layer continues to be thicker than the media.

At 1– months of age it is noted that a marked increase in the size of the lumen has occurred at the same time as the width of the wall has decreased. This results in a ratio between lumen and wall more than double that at birth. The adventitia does not decrease in width as does the media, so that the ratio M/A decreases.

In the subsequent development the ratios appear for the most part unchanged (Fig. _.) The already fairly sparse smooth muscle cells of the media diminish



Fig. 3

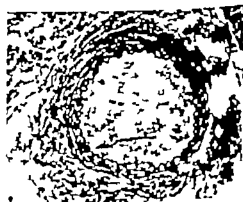


Fig. 4

Fig. 3. Ela[st]ic pulmonary artery of child 4½ years old. Lumen has increased and wall th[ick]ness decreased. Ratio lumen wall thickness high (15).

Fig. 4. Ela[s]to-muscular pulmonary artery of an infant days old. Media is dominated by smooth muscula[t]ure but in the middle [t]hird there are 3 concentric elastic band[s]. Lumen small, [w]all [t]h[ic]k. Ra[t]io lumen wall thickness low ( ).

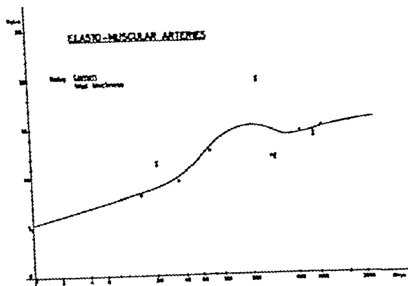


Fig. 5 Elasto-muscular arteries. Ratio lumen wall thickness

successively The adventitia decreases likewise in width, but continues to be thicker than the media (Fig 3)

Elastomuscular arteries As previously described these arteries are an intermediate form between elastic and muscular arteries

From birth until about two weeks of age the average size of the lumen varies between 100 and 200 µ The internal elastic lamina is thick and wavy while the external elastic lamina is thinner and in some places split (Fig 4) The media is dominated by smooth musculature but two to three rather coarse continuous elastic bands are found predominantly in the middle third in addition to shorter thinner more wavy elastic fibrils The average width of the wall varies between 20-40 µ The adventitia is composed of coarse collagen fibrils in a rather tight arrangement Between these are found short and thin elastic fibrils especially in the inner third but sometimes extending to the outer edge The average width of the adventitia varies between 45 and 5 µ and is thus thicker than the media Because of the comparatively narrow lumen and the thick wall, the ratio L/M is for the most part low about 4-8 (although in a few cases up to 11) (Fig 5).

Already from the age of one month, and definitely by 2-3 months the average size of the lumen has increased (Fig 6) The media's muscle layer dominates but seems to decrease somewhat to the advantage of the elastic fibrils. These as previously mentioned, are coarse and parallel, especially in the middle The decrease in the wall thickness together with the enlarged lumen, brings about an L/M ratio (8-22) of more than double the value at birth. The adventitia remains considerably thicker than the media.

During the following months essentially no changes occur The adventitia pre

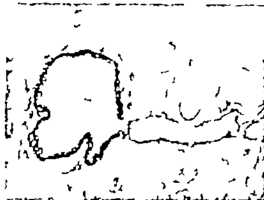


Fig. 6



Fig. 7

Fig 6. Elasto-muscular pulmonary artery of an infant 3½ months old. A muscular artery is seen branching off t the right Ratio lumen wall thickness high (17). 56.

Fig. 7 Larger muscular pulmonary artery of an infant 1 day old. The lumen is narrow and the wall thick. Ratio lumen wall thickness low (3.5). *02.

serves its greater width compared with the media the lumen is large and the wall thin.

Larger muscular arteries These arteries lie near those finer branches of the bron chial tree which still have columnar epithelium Their media consists of circu larly arranged smooth musculature The transition from elastomuscular arteries is gradual.

At birth and for some days thereafter the average size of the lumen is 35–80 μ. The intima s endothelium is evident and, especially in the smaller vessels the cells often extend out into the lumen Not infrequently the endothelial cells are loosened The internal elastic lamina is present in all vessels and, in the larger vessels, is thick and wavy (Fig 7) The musculature of the media consists of –4 cell layers In the larger vessels thin and short elastic fibrils are found especially in the middle and out to the adventitia Collagen fibrils in the media are absent. The external elastic lamina is distinct but in the larger vessels is not a thick as the internal elastic lamina and in some places is slightly interrupted. The adventitia which is thicker than the media, consists of coarse collagenous fibrils, often rather tightly arranged. In towards the external elastic lamina a few fine elastic fibrils are seen Because the wall is thick (average width 9–19 μ) and the lumen narrow the ratio L/M is low 3–4 (Fig 8).

At 2–3 weeks of age the lumen s average size (30–80 μ) is for the most part unchanged In vessel of smaller size the endothelial cells project out into the lumen. The internal and external elastic laminae are seen in all vessel but the latter continues to be somewhat thinner than the former and both are sometimes wavy In the media s musculature especially of larger but also of middle-sized vessels, thin elastic fibrils are seen The adventitia is now somewhat more loosely built than at an earlier age In the central third are found thin elastic fibril The ratio between the lumen s size and the width of the wall (L/M) is about –6

By –3 months of age the lumen s

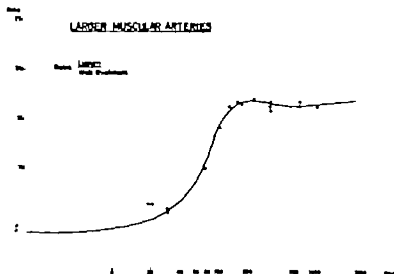


Fig. 8. Larger muscular arteries. Ratio lumen wall thickness.

average size has generally increased considerably and is now about 60–120 μ (Figs. 9 and 10). The media's musculature has become thinner and consists of 1–3 cell layers with a few thin elastic fibrils. The internal elastic lamina is fairly smooth and is as thick as the external elastic lamina. The adventitia is as before thicker than the media. The ratio between the lumen's size and the wall thickness has, because of an increased lumen and a thinner media, increased to about 9–16, thus 3–4 times more than earlier.

Subsequently there are essentially no changes, except that the lumen increases somewhat and the wall thickness decreases (Fig. 11). The adventitia remains constant in relation to the media, and is almost



Fig.



Fig. 10.

Fig. 9. Larger muscular pulmonary artery of an infant 1 month old. Ratio lumen wall thickness intermediate (10). 2.1.

Fig. 10. Larger muscular pulmonary artery of an infant 3½ months old. Ratio lumen wall thickness (14). 250.

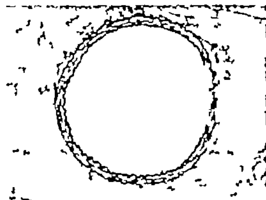


Fig 11 Larger muscular pulmonary artery of a child 5 years old. Lumen wide wall thin. Ratio lumen wall thickness consequently high (15). ×24.

tima project into the lumen and the cells are almost cuboidal. The internal elastic lamina is seen in most of the vessels, but as a rule is thin and interrupted. The media s musculature is composed of 1 ° cell layers. No elastic or collagenous fibrils are seen in the musculature The external elastic lamina is distinct in all vessels and i somewhat thicker than the internal elastic lamina The adventitia is composed of a relatively thick layer of collagen fibrils. No elastic fibrils are present in the adventitia The wall thickness is, on the average about 5–6 μ The ratio between lumen and wall L/M therefore is thus very low about 2 (Fig 14).

By °–3 weeks of age the lumen s size increases to about 10–°0 μ In addition, the elastic tissue has evolved further in the internal elastic lamina, which is now seen in all vessels and is seldom interrupted (Fig 15) The wall thickness is unchanged, but a few fine elastic fibrils have begun to appear The external elastic lamina is present in all vessels, but continues to be somewhat thicker than the

twice a thick. The ratio between the size of the lumen and the wall thickness is, as a rule between 1°–27

Precapillary muscular arteries These vessels constitute as mentioned previ ously the finest muscular vessel before the transition to arterioll and capillaries.

At birth and for some days afterwards the lumen s average size is 9–13 μ (Figs 1 and 13) The endothelial cells of the in

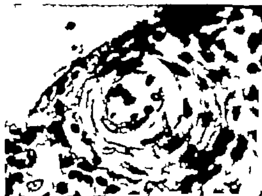


Fig. 1_



Fig. 13

Fig 12 Precapillary muscular pulmonary artery of a stillborn child. The endothelial cells of the intima project into the lumen Both lastic laminas are seen. Distinct cell nuclei of sm muscle in the media. Narrow lumen hick wall. Ratio lumen wall thickness low (3) ×

Fig 13. Precapillary muscula pulmonary artery of an infant da old Ratio lumen wall thickness 2. 1500.

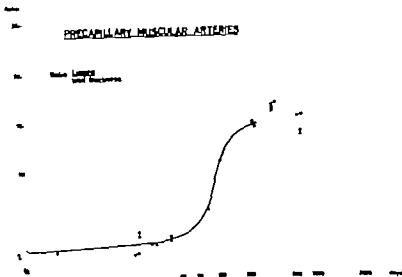


Fig. 14. Precapillary muscular arteries. Ratio lumen wall thickness.

internal elastic lamina. The adventitia's width is slightly greater than the media's, but is often rather loosely built. A few fine elastic fibrils begin to appear in the adventitia near the border of the media. As no changes occurred in the size of the lumen or the wall thickness the ratio between these is the same.

At ½–4 months of age a big and pronounced change occurs in the size of the lumen (Fig. 16). Its average is now between 10–15 μ. The intima's endothelial cells are thin and flat, in comparison with their more cuboidal appearance earlier. The internal elastic lamina is as a rule seen, but can be interrupted in a few vessels. The media is composed of only one layer of muscle cells which I occasionally



Fig. 15.



Fig. 16.

Fig. 15. Precapillary muscular pulmonary artery of an infant 1 month old. Ratio lumen wall thickness (2). 1103.

Fig. 16. Precapillary muscular pulmonary artery of an infant 3½ months old. Ratio lumen wall thickness (6). 1103.

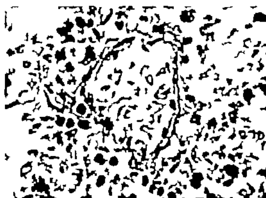


Fig. 1



Fig. 18

Fig 1 Precapillary muscular pulmonary artery of child 14 months old. Lumen wide all thin. Ratio lumen:wall thickness high (17) 88

Fig 18. Precapillary muscular pulmonary artery of a child 8 years old. Ratio lumen wall thickness (13)

interrupted, and has an average thickness if 2–4 μ The external elastic lamina is, as previously distinct in all vessels. It should be pointed out that both the internal and external elastic laminae can be distinguished, even if the musculature is interrupted. The adventitia is often composed of a fairly loosely built layer The small width of the wall, together with the increasing size of the lumen gives an L/M ratio which is notably increased to about 6–2.

The changes during the subsequent growth are relatively few (Figs. 17 and 18) The lumen, as a rule is about 40–60 μ but variations in both directions can occur The width of the wall is about 2–3 μ and the L/M ratio rarely is under 10 As a rule the adventitia is greater in width than the media.

Discussion

At birth and for a few days thereafter the pulmonary arterial bed (from the elastic arteries to the precapillary muscular arteries) has a characteristic appearance with narrow luminae and thick vessel walls. The media as well as the adventitia are thick and the similarity with the systemic arteries is great

During the first months of life there is a development in that the lumen becomes greater and the vessel wall decreases in width. This change reflected in the ratio between lumen and wall thickness takes place slowly at first is later accelerated, only to become slower again (Fig 19).

In the larger muscular arteries and the precapillary muscular arteries the size of the lumen increases and the wall thickness decreases rapidly especially during the first 3–4 months of life (Tables 1 and 2). Later these changes decelerate and after 8 months of age they have completely subsided. When comparing these pulmonary vessels and those of a 5 to 6-year-old child, there is no great difference

Regarding the elastic arteries the changes in the size of the lumen and the width of the walls occur earlier than in muscular arteries (Tables 1 and) The most accelerated development seem to appear as early as 1–2 months of age

TABLE 1 *Average values for lumen wall thickness and ratio in different ages (Different pulmonary artery types)*

Artery type	Age: Class boundaries (days)	Age: Class mid point (days)	N	Average values: Lumen, μ	Average values: Wall thickness, μ	Average values: Ratio Σ lumen/Σ wall thickness
Precapillary muscular arteries	0– 29	13	14	14.4	6.5	2.6
	30–119	57	13	22.6	5.6	4.5
	120–299	210	13	41.8	2.9	14.6
	300–749	494	13	45.5	3.0	15.3
	750–	1267	13	54.4	3.6	15.[illegible]
Total	—	—	66	—	—	—
Larger muscular arteries	0– 29	12	14	53.4	13.3	4.2
	30–119	57	13	83.4	10.0	8.3
	120–299	210	13	126.1	7.5	16.9
	300–749	494	13	1[illegible]7.3	7.8	16.4
	730–	1267	13	151.0	9.0	16.7
Total	—	—	66	—	—	—
Elasto-muscular arteries	0– 29	13	14	105.8	24.0	8.2
	30–119	57	13	[illegible]73.0	23.6	11.6
	120–299	200	11	331.5	1.9	15.1
	300–749	453	12	294.9	21.0	14.1
	750–	126[illegible]	13	418.8	27.3	15.1
Total	—	—	63	—	—	—
Elastic arteries	0– 17	7	8	489.6	56.0	8.2
	18– 44	27	8	600.6	52.3	11.5
	45–119	73	6	[illegible]3.3	42.2	14.3
	120–359	200	6	533.5	36.6	14.6
	360–	1360	6	709.2	48.3	14.7
Total	—	—	34	—	—	—

The elastomuscular arteries belong to an intermediat group not only from the morphological, but also from the developmental point of view for their greatest changes occur during –3 months f age

The anatomical changes are reflected in the hemodynamic behavior of the pul monary circulation during intrauterine perinatal, neonatal and the early periods of life

In the fetus, the ductus arteriosus serves as a connection between th right ventricle and the descending aort The pressure in the fetal pulmonary artery some what greater than the aortic pressure so that only a fraction of the blood which leaves the right ventricle goes to be pulmonary vascular bed (6) On the other hand the greatest part of the blood is propelled through the ductus arteriosus into the descending aorta and further to the placenta. This shunt is governed by high vascular resistance in the pulmonary circulation Thi high resistance is brought about by the fact that the lungs are collapsed The pulmonary arterial vessel during fetal lif how narrow luminae and thick vessel walls according to O'Neal *et al* (9) as well as Larroche Nodot & Minkowski (8) and thus resemble

TABLE 2. *Smoothed values for ratio lumen wall thickness at different ages. Change in ratio. (Different pulmonary artery types.)*

Age (days)	Precapillary muscular arteries: Ratio Σ lumen/Σ wall thickness	Change	Larger muscular arteries: Ratio Σ lumen/Σ wall thickness	Change	Elasto-muscular arteries: Ratio Σ lumen/Σ wall thickness	Change	Elastic arteries: Ratio Σ lumen/Σ wall thickness	C[illegible]
1	(2.1)		(3.5)		(3.2)		(4.2)	
10	[illegible].5	(+0.4)	4.1	(+0.6)	8.0	(+[illegible].8)	8.7	(
20	[illegible].8	+0.3	4.8	+0.	8.9	+0.0	10.3	
30	3.1	+0.3	5.6	+0.8	9.7	+0.8	1[illegible].3	
45	3.6	+0	7.0	+*1.4*	10.6	+*1.1*	14.4	
60	4.7	+0.6	8.7	+*1.7*	1[illegible].0	+*1.*[illegible]	15.3	
90	8.8	*4.1*	12.6	+2.9	13.7	+*1*	15.4	
120	1[illegible].5	+*3.7*	15.2	+*2.6*	14.6	+0.9	15	
150	13.	+*1*	16.[illegible]	+1.0	15.0	+0.4	15.0	
180	14.3	+0.8	16.	+0.5	15.1	+0.1	14.9	
210	14.6	+0.3	16.9	+0.2	15.1	±0	14.6	
240	14	+0.1	16.8	−0.1	15.1	±0	14.5	
270	14.9	+0.1	16.8	±0	15.0	−0.1	14.4	
300	14.9	+0.1	16.7	−0.1	14.9	−0.1	14.4	
330	15.0	+0.1	16.7	±0	14.7	0.[illegible]	14.3	
360	15.0	±0	16.6	−0.1	14.5	−0.2	14.3	
450	15.1	+0.1	16.4	−0.2	14.[illegible]	−0.3	14.3	
540	15.1	±0	16.4	±0	14	±0	14.3	
720	15	+0.1	16.4	+0.[illegible]	14.5	+0.3	14.4	(
1 080	15.[illegible]	±0	16.6	+0.2	14.8	+0.4	14.5	
1 440	(15.)	(±0)	(16.7)	(+0.1)	(15.[illegible])	(+0.3)	(14.6)	(

Italic figures represent greatest ratio change in different age groups.

the systemic arteries (which are adjusted for a circulation with high resistance).

This characteristic morphology of the larger and precapillary muscular arteries seems to be important in the maintenance of a high intrapulmonary resistance to blood flow in the fetus (2, 9). Another contributing factor i the architecture of the vascular unit of the fetal alveolar duct. Reynolds has shown that in the fetus the terminal arterioles give rise to twisted coiled capillaries which continue directly into connecting venules (10).

At birth the lungs inflate and normally fill up moment rily with air. With expansion of the lungs the aforementioned coiled vessel become extended and there is an opening up of a new capillary bed. This capillary plexus can accommodate blood and would account for the increase in the pulmonary arterial blood flow following the first breath. The decrease in the pulmonary vascular resistance after birth would be a result of the [illegible] tension of the coiled vessel (10). Th[illegible] pressure in the pulmonary artery declines and becomes les than that in the aorta. The flow of blood through the ductus arteriosus reverses it direction and now goes from the aorta to th pulmonary artery a shown by Adams & Lind (1). That the ductu continues to remain open and permits a left to right shunt to exist even some time after birth, has been shown

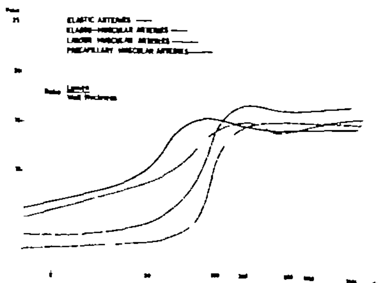


Fig. 19 Comparison of ratios lumen wall thickness of various types of pulmonary ... different ages. The changes in size of vessel lumen and wall thickness ... arteries than in muscular arteries.

by Rowe & James (12) with cardiac catheterization of newborn mongoloids without congenital heart disease and by Adams & Lind with catheterization of normal newborn babies. These authors could, with these examinations, demonstrate a left to right shunt through the ductus up to two weeks of age and an increased pressure in the pulmonary artery during the sam age period. Row & J mes had 6 cases below 14 days of age with pulmonary artery pressures between 30/10 and 80/50 mm Hg Adam & Lind demonstrated 6 of 7 cases below 70 hours of age having pulmonary arterial pressures be t een 31/10 and 50 10 mm Hg In addition, their result show that the pulmonary artery pressure ea ly a two weeks of age appears to be nea the ad lt value le ranging between 17/6–30 18 mm Hg, that is 20–25 of the systemic pressure

From this study w can demonstrate that the lumen widens ... of the vessel wall decreases ... months of life These ... more slowly during the ... they do later This de... be purposeful, for it ... prevent the occurrence ... right shunt through ... large shunt could ... an increased load on ... heart and subsequ... failure

After the first ... lumen and the thi... walls of the pul... occurs more qui... media undergoes ... After the fourth ... development ... and after the ... for the most pa... vessels of the ...

characteristic of the adult in whom the vascular bed is adjusted to a circulation with low resistance

Summary

The normal pulmonary arterial vascular bed undergoes a morphological development during infancy The intrapulmonary arteries at birth have narrow luminae and thick vessel walls, thus resembling the structure of systemic arteries During the first months of life there is a progressive change so that the lumen of the intrapulmonary arteries widens and the thickness of the vessel wall decreases. After 3–4 months of age this development takes place more slowly and after 8 months of life has almost subsided. In regard to the different types of intrapulmonary vessels, the elastic arteries (intrapulmonary arteries of greater size) seem to undergo an earlier development than the muscular arteries (smaller type of intrapulmonary arteries). Corresponding to the progressive vascular change during the first months of life the hemodynamic behaviour of the pulmonary circulation changes from the high-resistance circulation of the fetus to the low-resistance circulation seen in the adult

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References

1 Adams F H and Lind J Physiological studies on the cardiovascular status of normal newborn infants (with special reference to the ductus arteriosus). *Pediatrics 19* 431 1957

2. Brenner, O Pathology of the vessels of the pulmonary circulation. *Arch Int Med 56* 11 1935.

3. Civin W H and Edwards, J E: Postnatal structural changes in the intrapulmonary arteries and arterioles. *Arch Path, 51* 192, 1951

4. Dammann J F and Ferencz, Ch: Clinicoanatomic correlation in Henry Ford Hospital International Symposium on Cardiovascular Surgery Saunders, Philadelphia, 1955.

5 Dammann J F and Ferencz, Ch: The significance of the pulmonary vascular bed in congenital heart disease *Amer Heart J 5* 19 6.

6 D wes, G S Advances in Pediatrics Interscience Publishers, Ltd. London 1958.

Edwards J E. Functional pathology of the pulmonary vascular tree in congenital cardiac disease The Lewis A. Conner Memorial Lecture *Circulation 15* 164, 1957

8. Larroche, J C., Nodot A. and Minkowski, A.: Développement des artères et artérioles pulmonaires de la période fœtale à la période néonatale *Biol Neonat 1* 37 1959.

9. O'Neal, R. M., Ahlvin R. C., Bauer, W C and Thomas, W A: Development of fetal pulmonary arterioles. *Arch Path, 63* 309 1957

10. Reynolds, S R. M.: The fetal and neonatal pulmonary vasculature in the guinea pig in relation to hemodynamic changes at birth. *Amer J Anat 98* 97 19 6.

11 Rosen L., Bowden D H and Uchida, I: Structural changes in pulmonary arteries in first year of life *Arch Path, 63* 316, 1957

12. Rowe, R. D and James L. S: The normal pulmonary arterial pressure during the first year of life. *J Pediat 51* 1 1957

13. Wagenvoort C. T Torrielo, J and Thomas, W A: Structural changes in the pulmonary arteries exposed to high pressures from birth. *Arch Path, 57* 31 1954

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Respiratory Studies in Children

II. Functional Residual Capacity in Healthy Children

by F. GEUBELLE and P. DE RUDDER

Engström *et al.* ( ) have constructed a diagram representing the value of the lung volumes (residual volume V_R, functional residual capacity V_{FRC}, total lung capacity V_{TLC})[1] based on the regression of these values on the height measured on 93 children in the 6–14 year age group. The 95 % confidence interval around the regression lines are also given. When the lung volume of a single subject is being studied, it is possible to estimate the probability that the actual case belongs to a normal population.

The influence of training has been studied by Engström *et al.* ( ) on 80 double tests with an interval of 10–15 minutes. It has also seemed useful to determine the variability of the lung volumes during a longer interval, i.e. day to day, and to compare the variability with that found in adults.

With the method described by Engström *et al.* ( ) three problems have been investigated.

1. The accuracy of the method in our hands;

2. The magnitude of the physiological variations of the functional residual capacity (V_{FRC}) in healthy children.

3. The comparison between the physiological variation of V_{FRC} in children and adults.

Apparatus

Some differences are to be noted between our apparatus and that of Engström *et al.* (1) the spirometer capacity is 7000 ml; (2) the catharometer is manufactured by Kipp (Delft, the Netherlands) and the accuracy of the reading is 0.25 % between 0 and 20 %; (3) no membrane pump is used, but a diaphragm on the main spirometer circuit (after the fan type pump and the CO_2 absorber) shunt part of the expired air through the catharometer; (4) the fixed volume of the system, with the spirometer at its lowest position, is 7100 ml, (5) the helium concentration in the spirometric system before the determination is from 15 to 17 %.

All values are expressed in ml, corrected to body temperature, ambient pressure, saturated with water vapour (BTPS).

Material

(1) The volume of the two boxes, whose water capacities at room temperature were

[1] Terms as defined by a group of American clinical and research respiratory physiologists headed by J. R. Pappenheimer (3).

The fixed volume of the system is measured by the simple method described by Engström et al. (*). Fifteen determinations of this volume give a mean value of 7100 ml (range 7090 to 7130 ml).

1360 and 2540 ml, was each measured 10 times. The box was connected to the spirographic circuit in the same position as the subject.

( ) To study the physiological variation of V_{FRC} in children in the sitting position 69 duplicate determinations were performed on 23 apparently healthy children ranging in age from 6 to 15 years old.

(3) To study the physiological variation of the sitting V_{FRC} in adults and to compare it with that in children, 30 determinations were performed on 10 apparently healthy young adults (students, nurses, etc.)

Results

1 Error of the Method

Assuming the error in the water capacity volume recording to be negligible the random error of the spirometric method determined as the average of the random errors for the two boxes was 22 ml i.e. about 1 %. The error was obtained as the standard deviation of the repeated values Furthermore a systematic error exists which seems to make estimates of large volumes about 1 % too small. The mean estimated box values were significantly different from the known volume even though both errors were small.

2 Duplicate Determinations

The values of V_{FRC} are collected in Tables 1–3 Duplicate determinations were made on three occasions for each patient with an interval of some hours to several days. Individual differences between duplicate determinations at an interval of 15 minutes were calculated as the first minus the second determination (Table 4).

The errors of the method (equal to the standard deviation of the differences between duplicate determinations divided by the square root of two) are shown in Tables 5 and 6 The total average is [illegible] ml or 2.7 %. These errors are about twice those obtained with the boxes (22 ml or about 1 %)

3 Day to Day Variations

(a) The mean values of V_{FRC} (Table °) for the first the second and the third assay in each group do not differ significantly nor do the individual differences

TABLE 1 *Values of V_{FRC} (functional residual capacity) in 10 healthy adults. Time interval between (1) and ( ) is 4 days between (°) and (3) 6 days*

Height cm	(1) V_{FRC} in ml 1st	2nd	Mean	(2) V_{FRC} in ml 1st	2nd	Mean	(3) V_{FRC} in ml 1st	2nd	Mean
159	2364	[illegible]	2318	2234	2392	2373	2317	[illegible]	[illegible]
134	1868	1604	1931	17 9	1 01	1 14	1671	[illegible]	[illegible]
1 9	109	162	1650	14 1	1674	1 4	1943	191	[illegible]
160	1 3	1 31	1 6	1363	1518	1444	1 19	1654	[illegible]
16	184	1794	1910	[illegible]	[illegible]	[illegible]	13	15	[illegible]
165	131	11	2170	1705	1 44	1 65	1713	1 18	[illegible]
1 6	2719	[illegible]	[illegible]	3139	[illegible]	3193	3035	[illegible]	[illegible]
1 8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	1	43	3223	[illegible]	31 3	[illegible]	[illegible]	[illegible]
1 3	451		54	3209	3106	3157	[illegible]	[illegible]	[illegible]

TABLE . *Values of V_{FRC} (functional residual capacity) in eleven healthy girls T is the time interval between assays noted in hours (H) or days (D)*

Height cm	(1) V_{FRC} in ml 1st	2nd	Mean	T	(2) V_{FR} in ml 1st	2nd	Mean	T	(3) V_{FRC} in ml 1st	2nd	Mean	Age, years
104	625	670	650	4H	636	625	630	4D	704	648	675	6.5
129	696	730	714	4H	769	772	770	5D	696	757	726	11
145	1607	1612	1609	4H	1445	1447	1446	4D	1610	1700	1645	12
147	1241	1238	1240	4H	1109	1138	1123	4D	1310	1330	1320	12
149	1123	1106	1115	4H	1014	1049	1031	4D	1240	1263	1252	11
153	2110	2077	2093	4D	2098	2098	2098	4H	2490	2345	2417	15
154	1168	1145	1156	4H	1336	1304	1320	4D	1292	1283	1287	12
155	1806	1770	1768	4D	1893	1770	1881	4H	1640	1636	1643	13
158	1364	1414	1389	4D	1257	1243	1249	4H	1347	1307	1327	14
160	2321	2325	2323	4H	2190	2164	2167	4D	2180	2180	2180	14
164	1760	1730	1747	4D	1909	1892	1900	4H	2100	1930	2015	15

TABLE 3 *Values of V_{FRC} (functional residual capacity) in twelve healthy boys Time interval between (1) and (2) 4 days between (2) and (3) 6 days*

Height, cm	(1) V_{FRC} in ml 1st	2nd	Mean	(2) V_{FRC} in ml 1st	2nd	Mean	(3) V_{FRC} in ml 1st	2nd	Mean	Age, years
125	872	831	861	878	849	863	878	870	874	7
131	1178	1097	1137	1017	1067	1032	1040	992	1016	10
136	1094	1003	1049	1000	1080	1040	1062	1014	1035	11
137	1156	1171	1163	1250	1268	1259	1037	1136	1086	9
140	830	831	830	884	884	884	942	910	926	10
140	1214	1233	1223	1134	1118	1126	1215	120	1210	8
142	123	1243	1248	1263	1234	1250	1274	1229	1251	13
143	1164	1190	1177	1151	1169	1165	1109	1126	1118	11
149	1574	1530	1540	1460	1505	1492	1559	1516	1537	14
150	1527	1572	1549	1670	1630	1650	1522	1568	1545	14
160	420	2200	2310	445	2417	243	2390	2391	2390	14
1 1	496	2445	470	2439	4 1	430	2467	2521	494	14

TABLE 4. *Individual differences between duplicate determinations of functional residual capacity at three different period*

M.D mean difference E. error and S D standard deviation of differences.

	(1) V_{FRC} in ml M D	E.	S D	(2) V_{FRC} in ml M D	E.	S.D	(3) V_{FRC} in ml M.D	E.	S.D
Adult (16 cases)	62 2*	±25 9	8 1	22.3	±41.6	131.5	5	±10 8	34.3
Boys (12 cases)	23.2	20.9	2	0.3	±11	39	9.4	±10.3	35.6
Girls (11 cases)	− 1.3	± 9.5	31.6	24 1	±20 9	69 6	18 6	±25.6	84.9

Possibly significant All other values for M.D are not significant.

tions of other volumes will be compared with V_{FRC}. This basic information may well be useful in a day to-day study of the lung volumes in pathological conditions such as asthma or respiratory muscle paralysis and of the influence of drugs.

Summary

The functional residual capacity (V_{FRC}) was measured in ten normal adults and 23 normal children (11 girls and 12 boys) ranging in age from 6–15 years by a closed circuit method with helium as the test gas. Three assays, with some hours to several days interval, have shown a day to-day variation of about ± 6 in the three groups (± 5 % for the adult ± 3 7 % for the boys and ± 5.8 for the girls)

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References

1 Andrews, J F and Simmons, D H.: Lung volumes of normal and asthmatic children. *Pediatrics* 23, 507 1959.

2. Engström, L, Karlberg P and Kraepelien S Respiratory studies in children. I Lung volumes in healthy children, 6 11 years of age *Acta Paediat 45* 1956.

3. Pappenheimer, J R. *et al.*: Standardization of definitions and symbols in respiratory physiology *Fed Proc* 9 602, 1950.

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CASE REPORT

A Case of Ataxia Telangiectasia

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Louis-Bar described in 1941 (4) the syndrome later named after her of slowly progressive cerebellar ataxia and progressive symmetrical sclerocutaneous telangiectases, based on the case of a 9-year-old boy. In 1958 two cases were published by Centerwall & Miller (3). These authors succeeded in tracing 18 earlier cases, some published and some unpublished, and their series came to consist of 20 cases, 12 girls and 8 boys, from 12 families in Switzerland, the United States of America, England, and Holland. The first Scandinavian case was described in 1959 by Brandt (2) and in 1960 one was reported from Australia (Reye, 5).

The syndrome has been variously named Syndroma Louis-Bar, cephalo-oculo-cutaneous teleangiectases and, in the American literature, ataxia-telangiectasia. The following features are said to be typical. (1) Hereditary progressive ataxia commencing in early childhood. The ataxia has been described as being of cerebellar type sometimes with athetosis. (2) Symmetrically placed telangiectases on the eyeballs, face (sometimes with "butterfly wing" distribution), ears, the exposed areas of back and chest and hands. (3) Recurrent upper respiratory infections, including sinusitis, bronchitis, and bronchiectasis. (4) Indistinct, drawling, monotonous speech. (5) Delayed physical but normal mental development. (6) Dryness of the skin, thin hair with early greying.

Case Report

The patient was a girl born on July 14th, 1947. Her parents and 5 siblings were healthy and the parents are not related. There is no known family history of organic nerve disease or vascular disease. The patient was born at full term and weighed 3300 g and the delivery was normal. Development during the first year was rather slow but the child learned to walk by autumn 1948, when she had severe pertussis which lasted for 4 months. She stopped walking, but picked up again, and according to the parents behaved as other children up to the age of 4 years. She then developed slowly progressive difficulties in walking, and jerking and shaking of the arms and hands. She had great difficulty in keeping her balance with the upper part of her body but learned to ride a bicycle. The symptoms increased, particularly in the arms. When she started school, therefore she had difficulty in writing. On admission to this paediatric department in autumn 1955 her facial expression was stiff, and she had a slight intention tremor and light athetotic movements, but the reflexes were normal. She was assumed to have post-pertussis cerebral paresis, and was treated by physiotherapy. Some improvement was thought to take place at times.

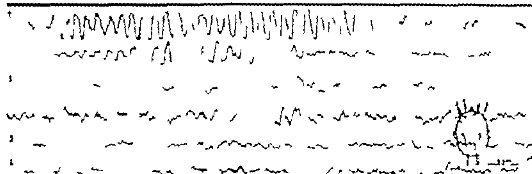


Fig. 1 The electroencephalogram on 13/2/1957 showing paroxysmal abnormality at the vertex.

The patient continued slowly to deteriorate however and by the age of 8 years she was unable to ride a bicycle

The child was admitted to another hospital in February 1957 She now had a rolling gait and placed her feet wide apart when walking but she did not fall. The finger to-nose and heel to-knee tests revealed intention tremor The facies was mask like and the conjunctivae were for the first time found to have telangiectases. The electroencephalogram showed a paroxysmal abnormality with a maximum at the vertex and a slight dominance at the left side (Fig. 1). The Wasserman and toxoplasmosis tests were negative Despite the progressive nature of the illness the diagnosis was still considered to be cerebral paresis subsequent to pertussis. The girl was treated at an institution for children with cerebral palsy for 8 months during 1957 1958, but deteriorated, and was discharged home Further deterioration took place and she could no longer either stand or walk without support She could dress herself and button up her clothes, but only very slowly owing to severe tremor of the arms and hands She received school tuition at home a few hours weekly and learned to read short words and to do simple addition and subtraction The capillaries of the face became gradually dilated, and later also those of the hands It is worthy of note that the patient according to her mother had never had a cold, and that she had been less susceptible to infection than her siblings



Fig. The patient in February 1960

In February 1960 the patient was readmitted to the paediatric ward at Västerås She was small and as can be seen from Fig. thin and round shouldered, with the head



Fig. 3. Telangiectases on the left eyeball and upper and lower lid.



Fig. 4. Telangiectases on the right hand.

projecting forwards. The arms, legs, hands, and feet were thin and trophic, with thin, dry cyanotic skin. Telangiectases were present symmetrically on the sclerae, eyelids, chin, neck, occiput and dorsa of the hands (Figs 3 and 4). She seemed absent minded but answered questions satisfactorily.

Uncontrolled, sudden, jerky movements of the arms and head took place occasionally, especially in connexion with voluntary movement and when the patient was concentrating. The facies was almost entirely expressionless, but on request she was able to produce smile or similar movements. There was loss f muscle power nd the musculature showed symmetrical trophy. The perception of light touch nd cutaneous pain as normal. There was marked ata ia with violent intention tremor and concomitant tremor movements of the other arm, head, and trunk. The child could not walk, even ith support and could n t stand unaided. The tendon reflexes were present but eak. Babinski sign was negat ve. The ocular fund were normal. The visual acuity was 0... on both sides (Monoyer scale). Skiascopy ga he following result right eye, 0 +1 D left eye 0 – +0.5 D. The ophthalmologist regarded the impairment of vision as being of central origin. It was not possible t carry out full vestibular investigation; the caloric reactions were normal however. Hearing was normal and so was the electroencephalogram. X ray of the skull showed a rather small skull-cap. Lumbar puncture was not done. Analysis of blood and urine ga e normal results. The intelligence quotient measured by the Terman Merill method was 43, although this seemed low with regard t the child development. Thi figure and the general impression gained of the patient were probably due t her motoric disorder and impairment of vision. The patient was discharged home without further treatment.

Discussion

The diagnosis of ataxia telangiectasia in this case is based on the progressive cerebellar ataxia, the patient appearance and the typically placed t langiectases.

A far as can be determined the

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CASE REPORT

Premature Synostosis in the Sternum (Silverman's Disease)

by S. BRÜNNER

From the Department of Radiology (Directors: K. A. Fine Licht and Olaf Petersen, M.D.) and the Pediatric Department (Director: P. Broestrup, M.D.), Copenhagen County Hospital, Gentofte, Denmark

Of the various congenital anomalies which affect the sternum, the most common is funnel chest. Premature synostosis is a much rarer sternal anomaly and is characterized by premature obliteration of the sutures between the sternal segments. This results in the characteristic deformity which is similar in appearance to pigeon breast.

The sternum is made up of the manubrium, four mesosternal segments, and the xiphoid process. Ossification of the centres in the manubrium and in the three proximal segments of the mesosternum usually begins during the last 3 or 4 months of foetal life, whereas the distal segment of the mesosternum and the xiphoid process generally do not ossify until after birth (2). Fusion of the different segments, which takes place from below upwards (Fig. 1), is completed between the 10th and 25th years of age, whereas the manubrio-sternal synchondroses remain unfused until after the age of 30 in 90 % of people (1).

Case Report

The patient, a 7-year-old boy, was the ... of ... children. Th... no family history of hereditary diseases or malformations, particularly no history of chest deformities. His birth history was normal. He was admitted for the first time at the age of 16 days, with a referred diagnosis of heart disease and was found to have Fallot's tetralogy combined with a patent ductus arteriosus. The patient was subsequently admitted seven times because of his heart disease, most recently at the age of ... in October 1959. Despite this he got on surprisingly well, developing almost normally for his age, although at times he had moderate dyspnoea and cyanosis of his lips. X-ray investigation as early as the age of 16 days revealed incipient synostosis of the sternum. Repeated X-ray films at the age of 6 months showed more marked changes (Fig. ...), and at 7 years showed fairly marked deformity consisting of pronounced forward angulation of the sternum (Fig. 3). Clinically, the chest was deformed, showing a midline bulge of the sternum (Fig. 4).

Discussion

Only ten cases of premature sternal synostosis have been reported previously, most of them by Currarino & Silverman (3). None was demonstrated in newborn infants, so that it cannot be stated definitely whether in some of the reported cases the sternum has formed from one ossifica-

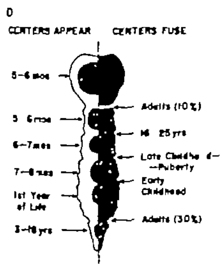


Fig. 1. The normal development of the sternum and ossification of the centres (after Silverman)



Fig. 2. Lateral roentgenogram of the chest of the patient when 6 months old. Note the forward angulation of the sternum and narrow sutures.



Fig. 3. The same pa[illegible] age of [illegible] Complete obliteration of [illegible] sutures [illegible] nounced deformi[illegible] of [illegible] um



Fig. 4. Photograph of the boy aged 7 years, with pigeon breast.

tion centre or whether the centres have fused after birth. Silverman mentions a patient, aged 3 days, who displayed slight forward angulation of the sternum with narrow sternal sutures. The youngest case on record was 1 month old (4). In the present case X-ray examination 10 days after birth showed that the sutures were narrow although segmentation was still present. The characteristic X-ray finding in all previous cases as well as in the present one is the fairly marked forward angulation of the sternum. There is no pathological evidence that the ribs or the diaphragm play a causal rôle in the premature fusion of the sternum.

It is remarkable that six of the ten previously reported cases have had congenital cardiovascular malformation, usually in the form of a shunt and signs of pulmonary stenosis. Since the present patient also has congenital heart disease it seems reasonable to think of a common disturbance in embryologic development. Premature synostosis of the sternum should be looked for in patients with congenital heart disease particularly those with intracardiac shunts or pulmonic stenosis. It may be more common than the literature would suggest.

Summary

A case of a rare sternal anomaly, premature synostosis, is reported characterized by premature obliteration of the sutures between the sternal segments. The clinical and roentgenologic findings are discussed.

References

1. ASHLEY, G. T.: The morphological and pathological significance of synostosis at the manubrio-sternal joint. Thorax 9: 159, 1954.
2. CUNNINGHAM, D. J.: Textbook of Anatomy. New York, Oxford University Press, 9th ed., 1951.
3. CURRARINO, G. and SILVERMAN, F. N.: Premature obliteration of the sternal sutures and pigeon breast deformity. Radiology 70: 532, 1958.
4. MOUNIER, J., GRAVIER, J., CHATEL, J. and LAMBERT, R.: Association d'une transposition vasculaire partielle (dextroposition aortique) a ce retour veineux anormal et d'une malformation thoracique par ossification prématurée du sternum. Pédiatrie 11: 93, 1956.

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CASE REPORT

Subperiosteal Haemorrhage in Haemophilia A and B

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In classic haemophilia (haemophilia A) as well as in Christmas disease (haemophilia B) severe skeletal anomalies may occur owing to repeated haemorrhages.

These anomalies are usually caused by repeated haemorrhages into the joints; the joints most frequently affected are the knees. Due to repeated haemarthrosis the synovia becomes hypertrophic and more vascularized, then the cartilage becomes affected causing irregularity of its surface.

Changes in the subchondral bone also take place, initially in th form of sclerosis and cysts If the intra-articular haemorrhages occur frequently the patient does not rest sufficiently and if no orthopaedic measures are taken nor measures for obtaining amelioration of the blood anomalies, then the deviations in the joint cartilage and in the subchondral bone increase steadily (compare *inter alia* Jordan (3) Middlemiss (4). This will result in severe malformations f the joints with impaired mobility and contractures.

Haemorrhages in the bone may also occur this may be in the subchondral part of the bone in connection with degenerative changes in the cartilage f the joint But the haemorrhages can also take place in the growing epiphysis or in the diaphysis of a long bone The latter may result in a picture which, for instance in the hip-joint resembles very much Perthes disease.

At first the bleedings in the shaft may give the impression of a bone cyst in which the necrotic bone is completely resorbed. But in this necrotic area calcification may also occur so that in the shaft a markedly calcified zone in the midst of an ischaemic zone is visible.

And finally a subperiostal bleeding may occur also this is a more rare occurrence. Occasionally a periostal reaction will be found in the neighbourhood of an affected joint as a symptom of reactive hyperaemia. In the subperiostal bleeding calcification may occur but later on resorption of the newly formed bone takes place so that the condition again becomes normal.

However occasionally a repeated subperiosteal and capsular bleeding may occur In this way a severe pressure is exerted on the underlying bone which may lead to pressure atrophy of the bone and later on also of the underlying bone-marrow

Usually a large firm swelling of the soft parts is present simultaneously in such cases we speak of a pseudo-sarcoma or of a pseudo-tumor in haemophilia. The development of such a pseudo-tumor depends on its localization and on the treat



Fig. 1. Patient R. Date: 5/3/59

ment. In principle there is no difference between the bone anomalies which may occur in haemophilia A and in haemophilia B, though cases of bone anomalies in haemophilia B are still relatively few.

We were able to observe such a pseudotumor in two patients, one suffering from haemophilia A, and in one with haemophilia B.

CASE 1. Patient R., a boy aged seven years and a half, is known to suffer from haemophilia A. Since he was one year old, he has been admitted repeatedly to our clinic, especially in the first years of life on account of haemorrhages of the skin, of frenulum and gums, but since his sixth year also because of repeated joint haemorrhages.

When six years and four months old he was admitted again, this time on account of an intense bleeding in the area of the right hip-joint. On admission he was very pale and showed a severe swelling from 5 cm below the right crest ... above the right knee. The ... in the right hip was greatly restricted. The haemoglobin content of the blood was ... In the next three weeks the boy ... of 750 cc blood and ... plasma, then the haematoma had become ... for the greater part and the hip could be moved slightly; however, a paralysis of the sciatic nerve developed. On account of an incipient contraction a suspension-stretch bandage was applied and one month after admission electrotherapy of the muscles of the lower leg was started. However, in the long run more adequate revalidation therapy was necessary and therefore the patient was transferred to the De Hoogstraat revalidation centre in Leersum. When the boy had been there for several months, he developed a large haematoma of the right heel, which within some days reached alarming proportions. Now the boy was transferred again to our Children's Clinic.

On admission the patient did not give the impression of being seriously ill; his feeding was excellent. Temperature 39.1° C. ... normal ties of eyes, ears, nose, mouth and throat, neck, breast and abdomen. A flexion contraction of the right hip and right knee was present. The right heel was enormously swollen and as big as the fist of an adult, as (Fig. 1). The skin ... red ... smooth and ... with increased venous pattern. There was ... equinus. The colour of the foot and of the toes was normal. The toes could move ... small. The X-ray ... showed marked ... rapid ... of the ... half of the ... calcaneus had disappeared (Fig. 1).

For the diagnosis we had to consider the possibility of haemorrhage a malignant tumour or osteolysis. BRUMMELKAMP (1) recently described the picture of pseudosarcoma in haemophilia and discussed the difficulties of the differentiation in these conditions with malignant tumours. VAN DE WEYER (5) at a meeting of the Dutch Orthopaedic Society communicated his experiences with two patients with *osteolysis* ("dit ementselle") a mysterious morbid picture in which, without any obvious reason, part of the skeleton totally disappears.

A diagnosis of osteolysis in a patient with haemophilia A was made The following therapeutical points had to be considered.

(a) prevention of decubitus of the highly stretched skin.

(b) promotion of the resorption of the enormous haemorrhage largely of old blood, by means of frequent transfusions of fresh blood or of fresh plasma (citrated or heparinized plasma) over a long period.

(c) prevention of new contractions.

(d) the already existing contractions had to be restored to a normal condition.

With reference to (a) the boy had to change his position in bed regularly In order to promote the resorption of the haemorrhage the boy received transfusions of 150 cc of heparinized plasma immediately after his admission, next day a transfusion of 500 cc of blood and from this day on daily transfusion of fresh citrated plasma (Van Creveld & Mochtar ( ) With regard to (c) and (d) the boy was encouraged to move all joint of the right leg actively

The further course was favourable Some days after admission the pain in the right foot abated considerably n th third day the temperature had dropped to



Fig .. Patient R. Date: 5/3 59

normal values and the swelling gradually became smaller After three weeks the skin over the heel was wrinkled, and the daily transfusions were discontinued.

After six weeks there was a considerable decrease of the swelling at that moment a distinct pes equino-varus as well as the persisting and still existing paresis of the nervus ischiadicus was present The poor position of the right foot was fixated and the roentgenogram showed a recalcification of the os calcaneum (Fig 3) Then the position of the foot was ameliorated by cautious manual redressement under protection of a plasma infusion, and the foot was placed in plaster Every two weeks this redressement was repeated, always under protection of a plasma infusion.

After three months a satisfactory position of the foot was obtained. No more haemorrhages occurred. The plaster-cast was replaced by a walking cast and the patient was mobilized

About five month after the admission, roentgenograms revealed a recalcification f the calcaneus though t form is somewhat different from the previous ones (Fig 4) the skeleton of the foot still shows some general trophy The contractures



Fig. 3. Patient R. Date: 15/4/39



Fig. 4. Patient R. Date: 1 /8 39

of hip and knee have disappeared. The neurological deviations have greatly improved, there is still a peroneal paresis, and on account of this the foot shows an inclination to become a club-foot. For this reason the patient again received a walking cast which later on was replaced by a leather support.

CASE 2. Our second observation is that of patient W., a boy with haemophilia B, now nine years old and only child of healthy parents. There are no haemorrhagic diatheses in the family. An intracranial haemorrhage occurred at birth.

During the first four years he often suffered from fainting fits, blue spots and nose-bleedings. Then no more haemorrhages occurred till at the age of seven years he fell with his right knee on a fragment of glass. The wound became infected and surgical intervention was followed by a haemorrhage in the right knee. The patient received a plaster cast for both legs and was admitted into the Children's Clinic for the first time. The haemarthrosis [illegible] treated by means of ice-bags, transfusions of blood and plasma and continuous nursing in plastersplints. The restricted extension of the right knee was completely restored to normal by means of a plaster stretch bandage. After a stay of about two months in the Children's Clinic the patient was discharged.

Some months later repeated haemorrhages occurred, later also in the right knee and a haemorrhage in the right heel developed. The roentgenogram of the foot revealed that the greater part of the calcaneus had disappeared. In consultation with the paediatrician (Dr. Wijffels) the boy, now aged eight years, was readmitted to the Children's Clinic.

The principal abnormality was localized in the right heel. Laterally and at the posterior of the heel a large and rather hard swelling, the size of a grape-fruit, was present. The skin of the heel was smooth and stretched. The roentgenogram of the calcaneus gave the picture as seen in Fig. 5. A talipes was present. The foot was placed in a suspension bandage in order to free the heel from any pressure. The patient was encouraged to make active movements with his feet. Then an intermittent infusion of fresh citrated plasma or lyophilized heparinized plasma was started and continued for 14 days. A week after the beginning of the



Fig. 5. Patient W. Date: 24/11/59.
Fig. 6. Patient W. Date: 12/2/60.

treatment the skin was less smooth and less stretched. The sensitivity was undisturbed and the toes could be moved in a normal way.

A month after his admission exercises under supervision of a physiotherapist were started. The swelling and discoloration gradually diminished and the regular check by means of roentgenograms showed an increasing calcification in the osteolytic area (Figs. 6 and 7).

Three months after the admission the boy started walking between walking bars with no weight bearing on his right foot. Two weeks later a haemorrhage in the right knee occurred which soon disappeared after several transfusions of plasma. Still some weeks later another haematoma occurred. Now the walking exercises were discontinued for some weeks, and about 7 months after admission these exercises were cautiously started first for some weeks under supervision in walking bars and after that with two crutches. In the meantime the regular roentgenologic control of the heel had shown an important improvement of the ossification (Fig. 8).

units in the course of two days and then went home. As roentgenograms two months later showed only slight improvement she was then given five drops daily of AFI D fort (7000 units per drop) and the dose was later increased to 70 000 units per day. Following this increased dosage her rickets healed satisfactorily. Serum Ca remained normal but serum P remained low (1.5–3 mg/100 ml), while the alkaline phosphatase activity showed some decrease although it was elevated most of the time. The Sulkowitch reaction in the urine was + or + +. She has continued to take vitamin D daily since the first hospitalization in dosages between 50 000 and 100 000 units. A supracondylar osteotomy was performed on both femora at the age of 11 because of increasing deformities of the lower extremities. At the age of 13 she had a grand mal seizure which lasted several minutes. EEG showed an active spike focus in the left temporal region and phenobarbital treatment was started.

The most recent follow up was when she was 14 years old. She now had a short and broad stature. Height 14_ cm (below the 2.5 percentile for age), weight 44 kg. The head appeared enlarged. There was bilateral exophthalmos. The thyroid gland was diffusely enlarged and she had a thyrotoxic appearance with tremor manus and clammy hands. There was slight acne. Pulse rate 150, BP 160/90. The gait was waddling. The lumbar spine was flattened and a thoracic lordosis was prominent. There were flexion contracture of 5° of both knees. Routine blood counts and urine analyses were normal. Serum chemistry: Ca 10.2 mg/100 ml, P 3.1 mg/100 ml, alkaline phosphatase 26.4 Bodansky units, urea 31 mg/100 ml, PBI 3.6 µg/100 ml and cholesterol 1__ and 1 16 mg/100 ml. Urea clearance was normal and the creatinine tolerance test showed 74.2 (normal 3_). Tubular reabsorption of phosphorus (TRP) 54 (normal ____). Urine paper chromatography showed slight but insignificant generalized amino aciduria and no definite ____ ____. Both TRP and chromatography were performed during treatment.) BMR 140 _, 150 _, 150 _.

The roentgenograms showed no sign of rickets. The epiphyseal lines were closed. The polyostotic lesions had progressed since the first examination. In the skull the lesions were now almost generalized and most marked around the left orbit. New lesions had appeared in the spinal column with compression fractures of T5, L4 and L5, and the ribs now revealed typical dysplastic changes. The lesions had also progressed in the extremities on the right side. Only the metaphyses and diaphyses were affected while the epiphyses were normal. An additional diagnosis of hyperthyroidism was made and treatment with Neomercazol instituted.

CASE 2. N. B., a girl, born 1951. The parents and her two siblings are healthy. An uncle has congenital luxation of the hip. There are no further known cases of congenital anomalies in the family and especially no other known case of renal glucosuria. Pregnancy, delivery and the neonatal period were normal. From 6 to 1_ months age she suffered from eczema and recurrent upper respiratory tract infections, had a poor appetite and was underweight. During the first year the psychomotor development was normal, later she appeared to be slightly retarded. At age 2 years she started limping and the right leg was found to be shorter than the left one. Later that year (Dec. 19_7) the left tibia was fractured, but healed in good position. In May 1958 the left humerus was fractured and she was treated in a local hospital. At that time it was noted that she had glucosuria with normal fasting blood sugar levels. Because of a ___ trochanteric fracture of the left femur in May 1959 at age 4½ years, she was treated in an orthopedic surgery hospital and then transferred to the University Pediatric Clinic for investigation of her glucosuria.

Physical examination. She was pale and tender and a little irritable. Height 10_ cm (1_ percentile for age), weight 14.4 kg (_ percentile for height). She had ___ little



Fig. 1



Fig. 2.

Fig. 1. G. B. (Case 1). Right radius and ulna. Fibrotic areas predominantly in the right ulna with normal bone structure between the localized lesions.

Fig. 2. X. S. (Case 2). X-rays of both lower legs. The left tibia shows fibrotic areas and scattered patches of irregular rarefaction. The right leg is normal.

skin, but no other discolorations. The left leg was 3 cm longer than the right one and showed a lateral bending.

Laboratory investigations. Urine sp. gr. 1.012, Heller neg., Clinistix +, Benedict reaction ..., Sulkowitch +. The positive reactions for reducing substance were found in most specimens, but an occasional specimen taken during fasting was negative. The reducing substance was proved to be glucose by chromatographic studies. Routine blood counts were normal. Fasting blood-sugar level was 85 mg/100 ml; glucose tolerance test began at 85 mg/100 ml, rose to 193 mg/100 ml at ½ hour and was [illegible] mg/100 ml after 3 hours. Serum chemistry: Ca was 9.9–10.1 mg/100 ml, P 2.2–2.7 mg/100 ml, alkaline phosphatase 10.5 Buch units, protein 6.7 g/100 ml with normal electrophoresis curve and thymol turbidity 0.06. Roentgenological examination of the skeleton showed changes characteristic of fibrous dysplasia in the extremities on the left side (Fig. 2), while the skull, ribs, spinal column and the right extremities were normal. The left leg was at least 4 cm longer than the right one. In the metaphyses and diaphyses of the affected bones there were areas of translucency and increased opacity alternating with bone of normal appearance. In the left femur a fracture line was noted. The affected bones were broader than normal. Intravenous

pyelography showed enlargement of the left renal pelvis *without signs of stenosis. The* calyces were broad and short

A diagnosis of f.d.b. and renal glucosuria was made and no treatment was begun. Because of increasing bending of the left femur a corrective osteotomy was performed in Oct. 1959 with a satisfactory result. From July 1959 to Feb. 1960 she was readmitted 3 times to the Pediatric Clinic for further studies. At the physical examinations she was unchanged except for the operative changes. The laboratory investigations showed glucosuria as before while the fasting blood sugar and glucose tolerance tests were normal on repeated examinations. Serum chemistry: Ca remained normal, units P ...6-4 mg/100 ml alkaline phosphatase 11.6-13.1 Bush units, urea 24-6 mg/100 ml, creatinine 0.5, 1.0-1.2 mg/100 ml, CO_2-combining power 48.8 vol %, chlorides 103 mEq/L, potassium 4.0 mEq/L. Creatinine tolerance test 66.6% and TRP 76%.

Two-dimensional paper chromatography showed a generalized pathological aminoaciduria in four of the five tested specimens. Most increased were leucine valine proline and phenylalanine but cystine, serine glycine alanine threonine and glutamine were also present in pathological amounts. Chromatography of plasma showed no abnormal amino-acid pattern. X rays did not show further changes.

Discussion

The diagnosis of f.d.b. is well established in both cases. The first case (G. B.) exhibited the classical triad of McCune-Albright's syndrome. In addition she had vitamin D resistant rickets and hyperthyroidism. N. S. Case No 2 has polyostotic fibrous dysplasia of the bones; aminoaciduria and renal glucosuria have also been found.

The symptomatology of f.d.b. is well known and will not be reviewed here. The three chief features of the disease are (1) skeletal lesions affecting only one, a few many or almost every bone in the body (2) pigmented patches in the skin, (3) endocrine disturbances manifested by sexual and somatic precocity especially in the female and other endocrine disturbances. The skeletal lesions are an essential feature and may or may not be accompanied by either or both of the other two (14). Hyperthyroidism has been reported in several cases (14), cardiovascular anomalies in two cases (14) and diabetes mellitus in one case (15). Symptoms and signs due to mechanical pressure on the eyes and spinal cord, as well as spinal and cranial nerves, are fairly common. To the authors knowledge the combination of f.d.b. and renal tubular defects has not been reported previously.

On her first admission to the hospital G. B. *had full-blown rickets. According to* the history she had received an adequate vitamin D intake and she did not respond to ordinary doses of the vitamin. Her serum phosphorus remained low during treatment and the alkaline phosphatase was elevated, although it fell somewhat with treatment. Further studies revealed a reduced TRP a finding which is in accordance with previous reports of vitamin D resistant rickets. Hyperglycinuria, an inconstant finding in vitamin D resistant rickets (4), was not present in our patient although it must be admitted that examination for aminoaciduria was not performed until after several years of treatment.

The ultimate cause of vitamin D resistant rickets is unknown. The low serum values and reduced tubular reabsorption

of phosphorus are constant findings. Several authors (4, 17) have stated that this renal tubular dysfunction is the probable cause of the disease However Albright (1) has suggested that the reduced reabsorption of phosphorus may be due to hyperparathyroidism (primary or secondary). The latter view was recently supported by Fraser *et al* (7) who studied the reabsorption of phosphorus during intravenous calcium load ing, and found increased reabsorption of phosphorus in the test period in the pa tients with vitamin D resistant rickets. As pointed out by Harrison in the discussion of Fraser s paper this does not necessarily exclude the kidneys as the site of dysfunction. Harrison suggests the possi bility of the renal tubules possessing in creased sensitivity to the parathormone in this disease (7).

The pathogenesi of vitamin D resistant rickets needs further clarification. W find it reasonable to believe that tubular dysfunction plays a significant role either alone or in combination with other metabolic defects. The finding of hyperglycl nuria in some of these patients may f vor this theory Inorganic phosphorus is re absorbed in the proximal tubules. Renal glucosuria and aminoaciduria are also believed to be results of reabsorption defects in the proximal part of the nephron (6). Accordingly the site of the renal defect in both of our cases of f.d.b can be located to the proximal tubules.

The fundamental biochemical mechanism in these tubular defects is not known, but the finding f Harrison & Harrison (0) that maleic cid inhibit the reabsorption of phosphate glucose and aminoacids, suggests that a common or losely related tubular transport system may exist for these substances. This is further supported by the occurrence of renal glucosuria, aminoaciduria and phosphaturia in syn dromes of apparently diverse origin such as cystinosis de Toni Fanconi syndrome lead (3) and lysol poisoning (18).

The diagnosis of renal glucosuria in our patient N S is based on the following findings. (1) Glucosuria which has been observed on different hospital admissions during 2 years. Glucose has been present in most specimens, but an occasional specimen taken during fasting has been normal. (2) Normal fasting blood sugars and normal glucose tolerance tests on repeated examinations. Simultaneously obtained urine specimens showed glucose in all but one Hyperglycemia has not been observed. (3) The reducing substance in the urine has been proved to be glucose by chromatographic studies (4) During the observation period of 2 years there has been no development towards a diabetic state

The present case does not fullfill all the criteria for the diagnosis of renal glucosuria stated by Marble (13) as some specimens have been normal. Others (10 11) do not believe that invariable glucosuria is a sine qua non for the diagnosis. Reubi (16) has forwarded the hypothesis that there are two distinct groups of renal glucosuria. In both groups there is a defect in the tubules, either separat or as a glomerular tubular imbalance The finding of hyperaminoaciduria in our patient also makes it likely that the glucosuria in this patient is due to a tubular defect

Luder & Sheldon (1) have described a family with four cases of renal glucosuria

pyelography showed enlargement of the left renal pelvis without signs of stenosis. The calyces were broad and short.

A diagnosis of f.d.b. and renal glucosuria was made and no treatment was begun. Because of increasing bending of the left femur a corrective osteotomy was performed in Oct. 1959 with a satisfactory result. From July 1959 to Feb. 1960 she was readmitted 3 times to the Pediatric Clinic for further studies. At the physical examinations she was unchanged except for the operative changes. The laboratory investigations showed glucosuria as before, while the fasting blood sugar and glucose tolerance tests were normal on repeated examinations. Serum chemistry: Ca remained normal, units P 2.6–4 mg/100 ml, alkaline phosphatase 11.6–12.1 Boch units, urea 24–26 mg/100 ml, creatinine 0.5, 1.0–1.3 mg/100 ml, CO_2-combining power 43.8 vol%, chlorides 105 mEq/L, potassium 4.0 mEq/L. Creatinine tolerance test 66.6% and TRP 76%.

Two-dimensional paper chromatography showed a generalized pathological aminoaciduria in four of the five tested specimens. Most increased were leucine, valine, proline and phenylalanine, but lysine, serine, glycine, alanine, threonine and glutamine were also present in pathological amounts. Chromatography of plasma showed no abnormal amino-acid pattern. X-rays did not show further changes.

Discussion

The diagnosis of f.d.b. is well established in both cases. The first case (G. B.) exhibited the classical triad of McCune-Albright's syndrome. In addition she had vitamin D resistant rickets and hyperthyroidism. N. S. (case No. 2) has polyostotic fibrous dysplasia of the bones; aminoaciduria and renal glucosuria have also been found.

The symptomatology of f.d.b. is well known and will not be reviewed here. The three chief features of the disease are (1) skeletal lesions affecting only one, a few many or almost every bone in the body (2) pigmented patches in the skin, (3) endocrine disturbances manifested by sexual and somatic precocity especially in the female, and other endocrine disturbances. The skeletal lesions are an essential feature and may or may not be accompanied by either or both of the other two (14). Hyperthyroidism has been reported in several cases (14), cardiovascular anomalies in two cases (14) and diabetes mellitus in one case (15). Symptoms and signs due to mechanical pressure on the eyes and spinal cord, as well as spinal and cranial nerves, are fairly common. To the authors' knowledge the combination of f.d.b. and renal tubular defects has not been reported previously.

On her first admission to the hospital G. B. had full-blown rickets. According to the history she had received an adequate vitamin D intake and she did not respond to ordinary doses of the vitamin. Her serum phosphorus remained low during treatment and the alkaline phosphatase was elevated although it fell somewhat with treatment. Further studies revealed a reduced TRP, a finding which is in accordance with previous reports of vitamin D resistant rickets. Hyperglycinuria, an inconstant finding in vitamin D resistant rickets (4) was not present in our patient, although it must be admitted that examination for aminoaciduria was not performed until after several years of treatment.

The ultimate cause of vitamin D resistant rickets is unknown. The low serum values and reduced tubular reabsorption

amino-acids. Arch Dis Child, 30 160, 1955.
13. Marble, A.: Renal glycosuria. Amer J Med Sc 183 811 1932.
14. Pritchard J E.: Fibrous dysplasia of the bones. Amer J Med Sc 222 313, 1951
15. Peck, F and Sa[illegible], C.: Diabetes mellitus associated with Albright syndrome Amer J Med Sc, 208 35, 1944.
16. Reubi, F C.: Glucose titration in renal glycosuria. Ciba Symposium on the Kidney p. 96. Boston 1954.
17. Robertson B. R., Harris, R. C. and McCune, D J Refractory rickets: mechanism of therapeutic action of calciferol. Amer J Dis Child, 61 948, 1942.
18. Spencer, A. G and Franglen G T.: Gross aminoaciduria following lysol burn. Lancet 1 190 1952.
19 Winters, R. W., Graham J B., Williams T F McFalls, V W and Burnett C. H., A genetic study of familial hypophosphatemia and vitamin D resistant rickets. Medicine (Balt) 37 97 1958.

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Toxoplasmosis in Children

A Study of 83 Swedish Cases

by GRETA HEDENSTRÖM, GUNNEL HULDT and RUTGER LAGERCRANTZ

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The interest in toxoplasmosis has been great ever since Wolf, Cowen & Paige (20) in 1939 described the first case in man. Pediatricians have primarily been interested in the congenital form of the disease. The clinical picture of congenital toxoplasmosis is well known. Severe cases show signs of generalized disease icterus, petechii, enlargement of liver and spleen and involvement of the central nervous system. Most reported cases have not presented symptoms of generalized disease, but instead have had one or several of the symptoms described by Sabin as typical of congenital toxoplasmosis (convulsions chorioretinitis hydrocephalus and intracerebral calcifications) Most cases have had a fatal outcome or a very poor prognosis with invalidizing symptoms and mental retardation. Gard Magnusson & Hagberg (4) and Hedenström (7) have however described cases with benign course and normal mental development

The acquired type of toxoplasmosis was first described in 1941 (1*) The cases which were initially described all had septic fever and many had a fatal outcome Later mild cases were observed and it seems that most of them ran a slight or a subclinical course. It is probable however that the disease always starts as a generalized infection, even though symptoms of this might be missing In Scandinavia the most common clinical symptoms seems to be lymphadenopathy with or without fever often resembling infectious mononucleosis (1 3 5 17 18). Few cases of acquired toxoplasmosis have been described in children. Sabin (14) was the first to describe a case in a child (1941), a six year-old boy who had meningo-encephalitis with a fatal outcome. Siim (16) has reported some cases with febrile lymphadenopathy in boys.

The great variations in the clinical picture of toxoplasmosis have thus been amply demonstrated. Yet the epidemiology is poorly understood. In planning a study in this field, we began with a survey of cases of toxoplasmosis diagnosed in Swedish children during the period 1951 to 1959

Material and Methods

The case material consists of 83 children with serological signs of an actual or recently

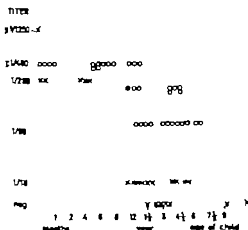


Fig. 1. Antibody titers in children with congenital toxoplasmosis related to age. O = titer in dye test, X = titer in complement fixation test.

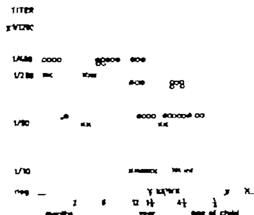


Fig. _. Antibody titers in mothers of children with congenital toxoplasmosis, related to the child' age. O = titer in dy test, X = titer in complement fixation test.

subsided toxoplasmosis. There was evidence that in none of the cases were the anti bodies demonstrated passively transferred. The cases came from various parts f Sweden, from Kiruna in the north to Lund in the south. Fifty-one children lived in urban and 33 in rural districts.

A summary of the clinical findings was obtained through the courtesy of the heads of various pediatric departments. Serological investigation using the dye test (DT) and the complement fixation test (CFT) were performed at the State Bacteriological Laboratory Stockholm (8, 15)

In the following presentation the material is divided int three groups: I congenital toxoplasmosis; II. acquired toxoplasmosis; III cases uncertain if congenital or acquired

I. *Congenital Toxoplasmosis*

The material comprises 62 cases of con genital toxoplasmosis The diagnosis in most cases was based on a typical clinical picture and elevated titer values for antibodies against toxoplasma. Fifty-six of these children had high antibody titers against toxoplasma (DT > 1/1250 and/or CFT > 1/120) The six remaining cases with lower titer values were all below one year of age, which indicated that the infection must have been rather recent and probably congenital. Fig. 1 illustrates all titer values obtained from these congenital cases and includes results of different measurements in the same patient. Blood specimens from the mothers were obtained in 49 cases and they all showed elevated titer values even several years after the birth of the patient (Fig. _).

Some of the findings in this study are summarized in Table 1 and Fig. 3 Fifty eight patients showed one or several of the symptoms described as typical of congeni tal toxoplasmosis. As these symptoms have been repeatedly reported in the literature we will not go into details here. Table 2 summarizes some findings in twelve patients observed during the neonatal period. These represent very severe cases with generalized disease and often fatal

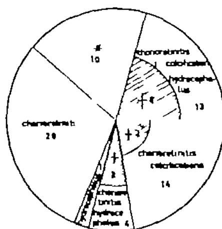


Fig. 3. Symptomatology and lethality in 62 cases of congenital toxoplasmosis. without chorioretinitis, hydrocephalus or intracerebral (i.c.) calcifications. Seven with epilepsia, eighth mentally retarded.

outcome. The following case might be men tioned as an example of this fulminating type of congenital toxoplasmosis.

H.G born Jan. 7 1959 Third hild of healthy parents. Some episodes of bleeding in the seventh month of gestation, born one month before term. Birth weight 2700 g At birth, the child had petechii and throm bocytopenia (min. value 1.,000/mm³) After a couple of days, the child's condition deteriorated she had a high pitched cry and developed generalized convulsions, a bulging fontanelle and icterus. The child had bilateral uveitis and X-ray of the skull showed calcifications around th ventricles. There was a marked anemia, bilirubinemia and gammaglobulinemia. The cerebrospinal fluid had very high protein content and in creased level of bilirubin and slight pleocytosis. Serological examination of serum revealed a DT of 1/1250 and CFT 1/120 The titers in the mother were at that time DT 1/1250 and CFT 1/480. Ten months later mother's DT was 1/1250 and CFT 1/120. Toxoplasma was isolated from cerebrospinal fluid collected on the ninth day of lif The urine was positive for protein but the sediment revealed nothing pathological. Inassears from the bone marrow no megacaríocytes were observed but the morphology was otherwise normal. The child's condition deteriorated in spit of treatment with sulfa merazin and aralen. He succumbed in the sixth week of life. Post-mortem examination showed very extensive encephalitis of the type repeatedly described in congenital toxoplasmosis.

In contrast to this fulminating type of congenital toxoplasmosis, the following case histories of two identical twins might be cited.

TABLE 1 *Symptoms and occurrence of prematurity and deaths among 62 patients with congenital toxoplasmosis*

Symptoms	Total number	Prematures	Dead	Epilepsia	Mentally retarded
Chorioretinitis Intracerebral calcifications Hydrocephalus	13	6	6	3	3
Chorioretinitis Intracerebral calcifications	14	2	2	7	5
Chorioretinitis Hydrocephalus	4	—	3	—	1
Chorioretinitis	20		—	4	7
Hydrocephalus	1	—	—	—	—
Without the bove symptoms	10	1	—	7	8
Total	62	11	13	21	26

TABLE Cl nical picture of congenital toxoplasmosis in the neonatal period

	Total number	Chorioretinitis	Intracerebral calcifications	Hydrocephalus	Enlargement of liver and spleen	High content of protein in liq. cerebrospin.	Lung symptoms	Toxoplasma organisms found
Surviving	5	5	5	3	1	4	—	—
Dead	7	7	7	5	6	6	7	7
Total	12	1	12	8		10	7	

TABLE 3. Condition at follow up in 50 surviving patie ts with congenital toxoplasmosis

Follow-up period, years	Number of patients with symptoms of: Chorioretinitis, epilepsia, mental retardation	Chorioretinitis, epilepsia	Chorioretinitis, mental retardation	Epilepsia, mental retardation	Epilepsia	Chorioretinitis	Mental retardation	No sequelae
1	—	1	1	—	—	—	1	—
1	1	1	—	2	1	4	1	—
3–4	—	1	1	5	2	3	4	1
5–6	1	1	3	—	—		3	1
–8	—	1	1	1	—	—	1	—
9	—	4	1	—	1	—	—	—
Total	2	8	7	9	4	9	10	2

Sachs Children Hospital, nos. 270/49 and 271/49. Boys, three years of age. Parents and two older siblings healthy. Pregnancy delivery and neonatal period normal. Normal development. Strabismus observed t the age of six and twelve months respectively. Examinations showed chorioretinitic scars—the mirror-image of each other in the right and left eye respectively. Otherwise the findings were normal. X-ray of the skulls were normal. The IQ was 91 for both boys (not tested under optimal conditions). Serology: one boy DT 1:100, the other DT 1:200 and the mother DT 1:200.

Fifty patients were alive when last examined. T ble 3 summarizes some of the findings at that time. It is probable that in most cases the toxoplasmosis was healed, but the residual symptoms were often severe. The mental retardation observed in 30 patients was of moderate degree corresponding to that of children who can benefit from teaching in special schools (IQ ca. 50–70, sometimes up to 80). Twenty four children were reported as having convulsions mostly of the grand mal type. Electroencephalography was done in five of these patients and showed evidence of grand mal epilepsy. The convulsive disorders seemed to respond to treatment in the usual way. At least 2 children had scars after chorioretinitis but in no case was the vision severely impaired on both sides. Most children had combination of several symptoms, which

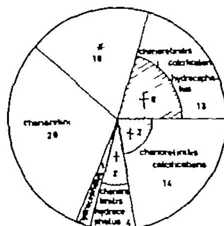


Fig. 3. Symptomatology and lethality in 62 cases of congenital toxoplasmosis, without chorioretinitis, hydrocephalus or intracerebral (i.e.) calcifications, Seven with epilepsie, eighth mentally retarded.

outcome The following case might be mentioned as an example of this fulminating type of congenital toxoplasmosis.

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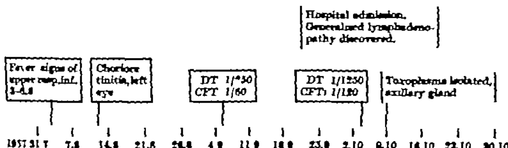


Fig. 4 Acquired toxoplasmosis in 10-year-old girl with chorioretinitis and lymphadenopathy Maternal DT and CFT negative

parasites were isolated from other areas of the patient. It therefore appears likely that the eye lesion was the result of ocular infection with toxoplasma.

In an eight year-old boy admitted to a department of child psychiatry a routine serological investigation revealed rising titers against toxoplasma (DT rose from 1/250 to 1/1250, CFT from 1/60 t 1/480). The boy was afebrile and had rectal bleeding. Blood examination showed anemia, elev ted gamma-globulin and slight eosinophilia (9%) and smears from the bone marrow reticulum-hyperplasia and increased number of eosinophile cells and plasma cells. Lumbar puncture and eye examinations revealed normal findings. Obviously this was a case of acute subclinical toxoplasmosis that was diagnosed occasionally

In eleven other children between 3 and 14 years of age, high antibody titers were found. The children had symptoms which seem not to have been caused by toxoplasmosis. Two cases are of special interest because the symptomatology was in some respects like that in toxoplasmosis

Two seven-year-old boys had lymphadenopathy and both had DT 1 1250 and CFT 1/120. They were first classified as cases of acquired toxoplasmosis. I one of the boy lymph node was removed which revealed evidence of t berculosis This diagnosis was verified by a guinea pig inoculation. In the other boy who, in addition to the lymphadenopathy had fever leucocytosis and en larged spleen, the Paul-Bunnell test was positive indicating an infectious mononucleosis.

In the last 12 cases the high antibody titers against toxoplasmosis *indicate* an acute or recently subsided toxoplasmotic infection, obviously of subclinical nature The cases demonstrate that care must be taken when correlating high titers, to any clinical symptoms that may be present

III. *Cases Uncertain if Congenital or Acquired*

High antibody levels were demonstrated in four patients in whom it was impossible to decide if the infection was congenital or acquired. Two children were four years old the others six and eight respectively The mothers had slightly elevated anti body titers. Two of these children had convulsive disorders one showed a slow speech development The last had strabismus and a behavior disorder probably of psychogenic origin. None of these patients had hydrocephalus intracerebral calcifications or signs of chorioretinitis.

Discussion

The diagnostic criteria in toxoplasmosis have been widely discussed (8 0 13 10) As in all infectious diseases the laboratory diagnosis should be based on the demonstration of the organism *and* a specific antibody response The isolation of toxoplasma is however difficult Material has to be obtained through biopsy (preferably from spinal fluid or involved lymph nodes) In direct smear or histopathological preparations it might be impossible to differentiate toxoplasma from other parasites. To isolate toxoplasma inoculation of mice is primarily used. It should be kept in mind that human strains are not always pathogenic to mice However specific antibodies always develop in the mice

It is thus often necessary to base a diagnosis on the results of clinical and serological investigations alone The clinical picture is not pathognomonic, as, e.g "inclusion body disease" may present the same clinical pattern as congenital toxoplasmosis (13) and mononucleosis sometimes cannot clinically be differentiated from toxoplasmic lymphadenopathy (1 8 18)

The specificity of the serological reactions seems beyond doubt after the vivid discussion of recent years (* 9). In newborns, elevated antibody titers which are not passively transferred suggest a congenital infection If the children are not examined until later in life it is often difficult to know whether the disease was pre or postnatal. In congenital toxoplasmosis, the mothers show elevated antibody levels for many years or even decades. If the mother is negative the infection in the child is probably acquired. If she is positive the child can have either a congenital or an acquired disease In differentiating between these two conditions attention should be given to the time of onset of symptoms as the same symptom can be present in both types (chorioretinitis and meningoencephalitis)

Significantly increasing antibody titers suggest actual infection. It is more difficult to evaluate unchanged high titers. The titers in the DT usually decrease within 6–7 months after an acquired infection but cases with titers remaining high and unchanged for years have been observed (8) In cases of unchanged high titers the diagnosis of an actual infection can be established only when there is a typical clinical picture with, e.g lymphadenopathy and typical changes in the blood and/or bone marrow and lymph nodes or when the parasite can be isolated from infected material.

Unfortunately there is no standardized procedure for serological investigations in toxoplasmosis and the antigens used seem to vary considerably (6) The titer values in this series are rather high compared with those of most other investigators. This is especially true of the titers in the CFT as earlier discussed by one of us (6). In individual cases, the titers in DT often are more constantly elevated and higher than those in CFT which correspond to earlier findings. This i however not true in some cases where the titers in CFT are higher than those in DT

Our findings in congenital toxoplasmosis (Tables 1 and *) resemble those in earlier series (4 8, 9 *0). Cases with neonatal disease usually run a severe and often fatal course (Table) In children who

developed symptoms later the most common was chorioretinitis but this was not present in all cases. The frequency of various symptoms is somewhat lower in this than in earlier series. We have in this study some mild cases also and two without residual symptoms. Such mild cases have earlier been reported (7) and might be more common than hitherto believed. We do not know why the clinical course was benign in these cases and can only speculat on the role of the infectious dose the virulence of the infecting organism and the protecting role of the transferred antibodies from mother to fetus.

In our patients with acquired toxoplasmosis two facts were of interest. Thus in an eight year-old girl with obviously acquired toxoplasmosis uveitis was the dominating symptom. Further 11 of 17 cases of acquired toxoplasmosis had a subclinical course which indicates that subclinical acquired toxoplasmosis is common in children as well as in adults.

As yet, no mother had two successive children with congenital toxoplasmosis. Thus there is no need to advise against further pregnancies as soon a there are no clinical signs of toxoplasmosis in the mothers and when the titers have begun to decrease

Summary

A report is given of 83 cases of toxoplasmosis in childhood. The series represents most cases diagnosed in Sweden during the period 1951 to 1950 The diagnostic criteria and the differential diagnosis between congenital and acquired forms of the disease are discussed. Sixty two patients were considered to have the congenital disease and 17 the acquired. In four patients it was impossible to determine if the disease was congenital or equired. The series of congenital cases comprises some with none or very few symptoms and normal development. Acquired toxoplasmosis in childhood seems to give the same clinical symptoms as in adults The most common symptom is lymphadenopathy with or without fever One patient had acute chorioretinitis The disease often runs a mild course and subclinical infections are probably not uncommon in childhood

References

1 ADAMSON C A., HEDENSTRÖM, G HULDT G and LIN[illegible]M, J Symptombilder på förvärvad toxoplasmos anslutning t ll 13 fall. Svensk Läkartidn 51 2717 195[illegible]
2. BAKAL, MA P Het Probleem van de Specificiteit van de Reactie van Sabin en Feldman bij de Diagnostiek van Toxoplasmose. Groen J J & Zoon, N V Leiden 1958
3. GARD, S and MAGNUSSON J H. A glandular form of toxoplasmosis in connection with pregnancy Acta Med Scand 141 59, 1951
4. GARD, S., MAGNUSSON J H. and HAGBERG B.: Congenital toxoplasmosis. Acta Paediat. 41 15, 1952.
5 GRÖNROOS, P Studies on toxoplasmosis and the serology of toxoplasmosis. A Med Exp Fenn, 33 310, 1955.
6 HA[illegible], H Toxoplasmose humaine mise en évidence des parasites dans les milieux intra-oculaires (tumeur acquérant exsudat rétro-rétinien). Arch Ophthal (Par) 14 470, 1954.
7 HEDENSTRÖM, G. On the variability of the clinical course of congenital toxoplas-

mosis with special reference to the occurrence of mild cases. *Acta Paediat* 46 579 1957
8. HULDT G The dye test and complement fixation test in toxoplasmosis. A comparative investigation. *Acta Pathol Microbiol Scand*, 43 141 1958.
9 — Acquired Toxoplasmosis. Human Toxoplasmosis. Munksgaard, Copenhagen 1960.
10. JACOBS, L., FAIR, J R. and BICKERTON J H.: Adult ocular toxoplasmosis. *Arch Ophthal*, 52 63, 1954.
11 KAYHOE, D E. JACOBS, L., BEYE, H. K. and MC CULLOUGH, N B.: Acquired toxoplasmosis; observations on two parasitologically proved cases treated with pyrimethamine and triple sulfonamides. *New Engl J Med*, 257 1247 1957
1_ MAGNUSSON J H.: Toxoplasmosens klinik. *Nord Med*, 45 344, 1951
13. PINKERTON H. and HENDERSON R. G Adult toxoplasmosis. *JAMA* 116 807 1941
14. Recent Advances in Paed. Ed. D GAIRDNER. J & A. Churchill Ltd, London 1958.
15. SA IN, A. B.: Toxoplasmic encephalitis in children. *JAMA* 116 801 1941
16. SABIN A. B. and FELDMAN H. A.: Chorioretinopathy associated with other evidence of cerebral damage in childhood. *J Pediat*, 35 296, 1949.
17 SABIN A. B., EICHENW LD H., FELDMAN H. A. and JACOBS, L.: Present status of clinical manifestations of toxoplasmosis in man. *JAMA*, 150 1063, 1952.
18 SAXÉN E. and SAXÉN L.: The histological diagnosis of glandular toxoplasmosis. *Lab Invest*, 8 386 1959.
19 SIIM, J CHR.: Toxoplasmosis acquisita lymphonodosa. Clinical and pathological aspects. *Ann NY Acad Sci*, 64 184, 1956.
20. WINBERG, P J Akut adult toxoplasmos med lymphadenopathi och chorioretinit. *Nord Med*, 47 563, 1952.
21 WOLF A., COWEN D. and PAIGE, B. H.: Toxoplasmic encephalo-myelitis new case of granulomatous encephalomyelitis due to a protozoon. *Amer J Path* 15 657 1939.

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SUMMARY OF SUPPLEMENT

A Longitudinal Study of Electrocardiographic Intervals in Healthy Children

by ROBERT W. McCAMMON

(*Supplement 126*)

Electrocardiographic interval measurements in the three standard limb leads were made on 4093 tracings taken serially from 14 healthy children from one month of age to their present ages as part of the study of human growth and development of the Child Research Council. Group standards were computed for P–R, Q–S and Q–T interval duration in the three standard limb leads and for pulse rate and Q–Tc in lead II. Individual subjects were presented against the group background to demonstrate developmental changes in healthy subjects from infancy to maturity. Characteristic patterns of stability and instability were discussed together with their clinical applications. The following conclusions were drawn.

1. Pulse rate is a very unstable measurement during at least the first two years of life becoming more stable after that but still showing both short-term and long-term fluctuations without obvious cause.

2. The P–R interval is a very stable measurement within the group range for any individual in health.

3. Changes in the P–R duration of more than 0.0? second on records from the same individual within any one year were not seen in the absence of illness or obvious change in pacemaker.

4. Changes in the duration of the P–R interval which occur in the absence of disease are usually confined to one lead, while those associated with cardiac abnormality occur simultaneously in all three leads to approximately the same degree.

5. No linear relationship could be demonstrated between P–R duration and pulse rate either for the individual or in the group data. A highly significant U-shaped dependence was found between P–R duration and pulse rate for the group data. It was speculated that this dependency resulted from some factor or factors not measured.

6. No relationship could be found between the duration of the P–R interval and heart size at a single age or between P–R duration and changes in heart size with body growth.

7. Group data for the Q–S interval duration strongly suggest that the duration of this interval is related to physical size and therefore to heart size though conformity to a pattern corresponding to established anatomic growth curves is not universal.

8. The frequent occurrence of Q–S durations in excess of 0.10 second after 1 years of age makes this an unacceptable upper limit of normal duration.

9 The Q–T interval shows partial dependence on the pulse rate both for the group data and within the individual, but this is not uniform or predictable

10 The corrected Q–T interval, using the square root ratio of Taran, shows as wide a range for the group data as is found for the uncorrected Q–T

11 Individuals in health characteristically fluctuate widely through the group range for both the Q–T interval and for the corrected Q–T interval.

12. The range for the corrected Q-T interval duration extends upward far enough so that a minimum of 5 % of the data falls above the previously published upper limits of normal.

13 The equation used for correcting the Q–T duration for pulse is inadequate for very fast or very slow pulses

14 There is a temporary rise in Q–T duration between the ages of 18 and 22 years which occurs in most if not all subjects independent of rate changes.

Acta Paediatrica 50: 315—324 March 1961

PROCEEDINGS OF PEDIATRIC SOCIETIES

Swedish Pediatric Society

Meeting Sept. 30 1960

Trevor P M Neonatal cold injury due to accidental exposure to cold

In Great Britain neonatal cold injury is a not uncommon disorder with a high mortality. In Brighton in the last six years or so 16 examples have been admitted to the Children's Hospital and no less than eight have died. Cases generally arise after home confinements in winter when outside air temperatures fall below freezing point. An unknown but appreciable number of newly born babies die in this way each year. This waste of life, which is almost entirely preventable, is now receiving considerable attention. The nature of the disorder frequently goes unrecognised, the predominant aetiological role of exposure to cold being overlooked. The condition may be incorrectly labelled sclerema neonatorum or haemorrhagic pneumonia, to mention two recurring misdiagnoses. Increasing apathy and food refusal are the most constant presenting features, but the infant does not look ill. In a number of cases coldness to touch has been noted for several days by the mother but she may have difficulty in arousing the interest of her medical attendants in this important physical sign. In none of the series has the severity of the hypothermia been realised before admission. Oliguria is a frequent accompaniment. Constant clinical findings are severe hypothermia (all cases below 32°C on admission; three less than 27°C), oedema of the extremities and striking skin erythema, the latter largely contributing to the misleading appearance of well-being. A purulent nasal discharge occurs commonly but sometimes not until rewarming is under way. The predominance of male infants in this series is striking (12 of 16 cases, males). Prematurity is not important there being only three small babies with birth weights of 5½ pounds or less amongst the 16 cold babies. Twelve of the 16 cases were admitted in January, February and March, generally the coldest months of the year. A noticeable feature is that the occurrence of cases generally coincides with spells of severe weather when minimum air temperatures fall below freezing point. Social conditions are not always bad; a comfortable and well-provided home may still be cold in winter, especially at night-time. Predisposing factors include asphyxia at birth, birth injury and infection. Food refusal and bathing in a cold environment play a contributing role in a frigid home. A prognosis is usually possible within a few hours of commencing rewarming and these are all considered ominous signs: apnoeic attacks, persisting oliguria, hardening of oedema and bleeding from the mouth and nose, the latter usually being accounted for by pulmonary haemorrhage.

A post mortem examination was carried out on the fatal cases. There were no specific features to relate an infant's death to cold injury. If at necropsy massive pulmonary haemorrhage, mild ascites and oedema of the extremities are found, then a careful scrutiny of clinical data is desirable for features suggestive of cold injury, especially during the winter months.

The best method of treatment is still undecided. Therapy may be considered under three headings.

Re-warming. The majority of cases fall into the chronic or prolonged group (hypo-

thermia for over six hours) for which slow or passive rewarming is favoured. This is best carried out by obviating heat loss and allowing the infant to warm up through the processes of met bolism.

Feeding One of the first effects of rewarming is to accelerate metabolism and to increase demands for glucose. Profound hypoglycaemia, if not present initially may be precipitated if nourishment is withheld. Where the hypothermia is prolonged as just defined, 15% glucose by the intragastric is being tried.

Drugs. As an infective process may be difficult to recognize initially and as septic manifestations often appear during resuscitation, antibiotic therapy should be given early Cortico-steroid treatment is advocated by some workers, but the rationale is not understood. Antibiotic cover is imperative if this hormone is used.

Meeting Dec. 2 1960

Karl Magnus Herrlin Frequency and prognosis in flexor spasms of infancy

At Kronprinsessan Lovisas Barnsjukhus during the ten year period, 1950–1959 693 children (390 boys, 303 girl) under the age of four years were examined because of epilepsy Of these 48 cases (26 boys, — girls) had flexor spasms ("Nick und Salaamkrampfe" spasmes en flexion") In this series of 693 children, °90 had the onset of seizures before one year f age 45 of whom (15.5%) had flexor spasms. Of all the 48 infants with flexor spasms, 19 (40 %) have died, 21 (44%) are severely mentally retarded and 8 (16%) are moderat ly retarded. The case material will be published.

Å Gyllensvärd and *S Malmström* *Cerebrospinal fluid in premature infants*

Studies of the cerebrospinal fluid with regard to cells, protein and bilirubin were performed in thirty-six prematures. The babies general condition their hemoglobin red and white blood cells, serum protein and serum bilirubin were also followed. Lumbar punctures were generally made on the third eleventh and thirty-ninth days of life (I II and III respectively in the table) The mean birth weight was 201 g. On the average, two of the tests from each child could be used for evaluation (in ten cases all three). The results regarding cells and protein are shown in the f llowing table. Cell content was not correlated to the birth weight while protein was negatively correlated. \ correlation was shown between the bilirubin in the serum and that in the spinal fluid. Nor between the general condition and the level of serum bilirubin, content of cells or protein. The highest value noted for the bilirubin was 19 mg %.

E. Rabe Tripl vaccination, methods and secondary effects

The most suitable area for a subcutaneous injection has been investigated in several groups of °00 children who received their first and second injection in tw different sites. The mother s opinion as t which injection caused the child and the mother the most trouble and anxiety has been evaluated. The majority of mothers regard the second injection as causing more trouble than the first one This source f error was voided by alternating the site f the first injection in every ther child. The best sit of injection was the pectoral region If the incidence of complaint is put t 1.0 for injections over the pectoralis muscle it is 4.. in the supra-

Puncture	I no. = 21 Cells/3 mm³	Protein/mg %	II no. = 23 Cells/3 mm	Protein/mg	III no. 23 Cells/3 mm	Protein mg
Mean	27	150	°0	110	17	84
Median	22	149	15	99	12	6
Spread	4–112	67 202	3–66	74–159	2 0	53–106

spinatus fossa, 2.6 in the deltoid region and 1.7 in the gluteal region, which thus is the second best spot of injection. The subcutaneous and intramuscular injections in the gluteal area were compared in one experiment. Intramuscular injections caused considerably more trouble than subcutaneous ones. Thus, 49 of 200 children had no complaints after the subcutaneous injection, while only eight had no trouble following the intramuscular injection. In a smaller series, shallow and deep subcutaneous injections were compared. After 4–6 weeks, a palpable infiltrate was noted in 40% of the shallow injections but only 3% of the deep ones. Abscess formation was 10 times more frequent after the shallow injection, which therefore ought to be avoided. Finally subcutaneous injections of Cutter vaccine and Swedish triple vaccine were compared. The incidence of complaints was equal.

DISCUSSION *H. O. Mossberg*. The mothers in Västervik were somewhat hesitant about triple vaccination in 1956 when the well baby clinic was started in the hospital there. Some unusually strong local and general reactions to the ordinary dose of 1 ml of vaccine were observed. Since 1958 the first injection of 0.7 ml has been given (deep subcutaneously supra spinam), the second and the third injections have been with 1 ml, or somewhat less, if the reaction to the previous injection had been too strong. The case material comprises slightly more than 300 children. Two injections have been given to 97.8% of the children, and three to 92.5%. Moderate reactions (i.e. fever 38 C for 2–3 days) possibly some sluggishness and local tenderness were observed after the first injection in 4%, after the second in 17.7% (78% injected with 1 ml) and after the third injection in only 5% (73% given 1 ml). Total frequency of moderate reactions was 8.9%, which is higher than earlier reported in any Swedish series. A dose of 0.5–0.7 ml has given local abscess formation in 0.8% compared with a frequency of ..4% following the dose 0.5–1 ml. Of the individuals with slight general reactions 1.3% developed abscesses, but 7.1% of those with moderate reactions later had abscess formations. It is probable that these doses convey somewhat lower immunity than full doses but the lower frequency of side effects might favour my approach.

B. Eckerberg. A case of prenatal mycosis

The patient, an infant girl, six weeks premature weighing 2800 g, was born after a normal delivery. On the second day of life she developed convulsions and on the fourth day diarrhea. On admission to the pediatric ward the sixth day she was sluggish, microcephalic and had a fluctuating tumor in the neck, the size of a walnut. Puncture of the tumor revealed pus from which *E. coli* were cultured. The patient later developed profuse diarrhea, vomiting and convulsions and died on the 25th day of life. On post mortem examination a cystic abscess was found connected to the esophagus and containing fungus and bacteria. Intracerebral calcifications were seen in the atrophic brain parenchyma, especially around the ventricles. Fungus colonies with appearance of *Candida albicans* were also demonstrated in the periphery of these calcifications. No fungi were found in other organs. This is probably a case of early prenatal infection with *Candida albicans*. It probably started as an esophagitis with abscess formation and later hematogenous spread to the brain. This case is analogous to one described by Burry in 1957 (*Arch. Dis. Child. 32*, 161).

C. Lingen, B. Wengle and *H. Boström*. Metabolic screening of urine for inborn errors of metabolism in pediatric material

During the last year a series of simple chemical reactions and paper chromatographic methods have been applied to urine specimens from all patients (1500) of the pediatric department of Karolinska sjukhuset, Stockholm. Only two cases of "inborn errors of metabolism" were found; one case of porphyria and one of cystinuria. This agrees well with the expected low incidence of these disorders. Some interesting facts about the excretion of hydroxyproline and of ethanol

amine together with beta-aminoisobutyric acid combined with lactosuria were observed. Thus an increased excretion of the aminoacid hydroxyproline was found in 50% of cases with hyperbilirubinemia not associated with immunological factors. Patient with hyper bilirubinemia caused by blood-group in compatibility (Rh or ABO) did not seem to excrete pathological amounts of hydroxy prolin

Symposium on poisoning

Introduction.—Bengt Karlsson Poisoning cases of current interest

During 1960 a Poison Control Center was established at the pediatric clinic Karolinska sjukhuset Stockholm Although the center has functioned primarily as an information center it is also concerned with education, research and treatment. The cases presented here have been selected to illustrat poison ing problems which have been referred to the center

Bengt Karlsson Saffron poisoning

An 18 year old woman was admitted to the Visby hospital 15 hours after she had st rted to complain of abdominal pain with diarrhea and vomiting She was in poor con dition with icteric skin and sclerae and with cyanot c lips. Blood pressure was 160/110- pulse 1*0 The abdomen was distended and there was a marked tenderness under the right costal margin Laboratory findings included Hbg 40%, Rbc 1.5 million, Wbc 20,800 hematuria. The patient became anuric and later she became unconscious but before that confessed that the evening before ad mission she had eaten saffron because of a suspected pregnancy She died a few hours after admission Post mortem examination revealed generalised icterus, pronounced pulmonary edema and swelling of the pa renchymatous organs. The histopathology howed pronounced renal tubular damage Saffron has been used for many years as an bortion provoking gent The lethal dosage is said to vary between 3 and 12 g

C. The én. Poisoning by furniture polish

A 1½ year old boy drank an unknown quantity of furniture polish. Half an hour later the boy vomited and the mother provoked further vomiting. He later became sluggish, hypotonic, and tachypneic When he arrived at Kronprinsessan Lovisas Chil dren s Hospital 1½ hours after the ingestion, he was unconscious, pale cyanotic and pulseless. He was immediately put in the respirator but the cyanosis persisted in spit of adequat ventilation ECG showed shifting pace-maker ventricular extra sys toles, and progressive increasing lowering of ST T segment as in severe myocardial ische mia. Later bradycardia developed hich changed into ventricular fibrillation following which the patient died. Post mortem exami nation revealed signs of moderat amount f aspiration of small hemorrages in the bronchi. Routine chemical analysis for poisons in the stomach contents, the blood and various organs was negative

Two-thirds of all the many different furniture polishes contain .5–75% vanolen, a petroleum derivat ve resembling kerosene In this case, it contained 17% vanolen, 3% xylol, 30% spikler oil, 1% ricinic oil, 2-3 aromatic oils and water

The polish was tested for t xicity at the State Pharmacological Laboratory It wa atoxic to mice when given orally in dosages up to 18 ml/kg body weight. Inhalation of pure vanolen and the ctual polish by mice and guinea-pigs in mist chambers did not cause any lung lesions.

Injection of kerosene in the trachea has, however been shown t cause death in a couple f minutes through hemorrhagic edema and membrane formation in the lung alveoli. Pneumonia after kerosene intoxica tion has been known for a long time and has been demonstrated by X ra examination after one hour Aspirat on of kerosene in animals and men has been shown t cause lung lesions. In text books th provoking of vomiting and the use f emetics after ke rosene ingestion is emphatically warned against Stomach lavage should be done

only after intubation. Olive oil can be given orally to decrease the resorption.

Propaganda for proper storage in the home of all cleaning fluids is the most important factor as the campaign for "protection seals" has been unsuccessful. The kitchen should be furnished with special cupboards which are out of reach of small children.

S. P Fällström. Jetex

A 2½ year old boy was brought to the pediatric clinic of Gothenburg one hour after eating one or two "Jetex" tablets. On the way to the hospital he had general convulsions. On admission he was unconscious, hypertonic and had increased tendon reflexes. Respiration was shallow and slow and respiratory arrest soon developed. He was treated symptomatically and he breathed spontaneously after three hours. The next day he was completely asymptomatic. "Jetex" tablets are used for combustion in toy motors and contain about 0.75 g potassium bichromate. This, however gives other symptoms of intoxication than those which the boy presented. The tablets also contain combustible substance of unknown nature which probably caused the intoxication.

B. Vahlquist. Pica of match heads

My case is rather unusual. A 14-year-old girl had been consuming match heads in increasing quantities over a period of several months—in the end several boxes a day. On admission to the Department of Pediatrics, Uppsala (record no. 184/60) she showed signs of grave anemia, with a hemoglobin level of 5.8 g per 100 ml., a red-cell count of ~1 million, and a colour index of 0.9. Clearly there was a combination of severe iron deficiency (serum iron 8 gamma %, bone marrow iron extremely low) and potassium chlorat poisoning. After the patient had stopped eating match heads and had received iron therapy the red-cell count rapidly rose and the hemoglobin level slowly improved with the result that the colour index fell to 0.5—a value typical of simple iron deficiency anemia. Six months after the end of the iron therapy the symptoms of pica began to appear again. Examination disclosed recurrence of the anemia, this time with a picture of simple iron deficiency. A box of Swedish matches contains 0.3–0.5 g $KClO_3$, and in addition small quantities of K_2CrO_4, $KMnO_4$, sulphur and glue. Moeschlin gives the acute toxic dose of $KClO_3$ for adults as 5–10 g. The daily consumption of up to 0.5 g or more for several months might well produce serious toxic effects. Iron deficiency anaemia is a common cause of pica. It should always be excluded first in older children and adults, since other causes (apart from pregnancy!) are less common. In young children pica involving a variety of substances may almost be regarded as a normal phenomenon during a certain stage of development.

R. Tunell and B. Persson. Chronic vitamin A intoxication in infants

About 40 cases of chronic vitamin A intoxication in infants, children and adults are reported in the literature. The dosages have in general been high (150,000–500 000 IU/day) and the duration of overdosage long (at least ½ year with few exceptions). For these reasons a report is given of three infants, each of whom received vitamin A 25,000 IU/day during a period of 1–3 months with symptoms of vitamin A intoxication. *Symptoms and signs.* History of sudden onset of hyperirritability, tenderness and edema in the occipital region, signs of increased intracranial pressure (diastasis of the sutures, bulging fontanel, sunset sign) and pronounced craniotabes were present in all three infants. All had an infection. Two of the infants had typical desquamation of the skin on the palms and soles. One infant had hypertonia. *Laboratory data.* Two cases had anemia, 2 cases had high values of alkaline phosphatase. All three infants had typical X-ray changes of rickets, none had cortical hyperostoses. All had normal liquor. Fasting vitamin A blood levels were obtained 3 days after vitamin A had been discontinued in one infant and 20 days after discontinuation in another. These values were 38 IU/100

ml and 410 IU/100 ml serum respectively. The upper limit of normal is 180 IU/100 ml. This examination was not performed in the third case. *Dose and duration.* All received 25,000 IU/day as 30 drops of "AD-vimin Astra" 1 month in one case and for 3 months in the other two. In cases the dose had been prescribed by a physician for treatment of craniotabes; the overdosage in the third child was the result of carelessness by the mother. *Course.* All symptoms disappeared when the vitamins were discontinued.

Comments. The manifestations of vitamin A intoxication in this age-group are characteristic except for the X-ray evidence of rickets which has not been previously reported in man. However rickets has repeatedly been induced in animal experiments and is said to depend on antagonism between vitamin A and D. The importance of care in prophylactic treatment of infants with water-soluble preparations of vitamin A is stressed.

Meeting Dec 3 1960

Guest lecture by *R. Debré, Paris.* Quelques études sur le sommeil de l'enfant et ses troubles.

Symposium. Swedish help to children in economically underdeveloped countries. Moderator A. Wallgren. Participants: M. Leysner, S. Heppling, G. Herlitz, O. von Sydow and B. Strindberg. Discussion: J. Asplund and M. von Malmborg. (Will be published elsewhere.)

Meeting Jan 13 1961

Jens Bergstedt: Does Xantocid have any effect on the streptococcal flora in the throat?

During the spring of 1960 at Stockholm's Hospital for Infectious Diseases, a clinical bacteriological investigation was done to determine the effect of the preparation Xantocid on the β-hemolytic streptococcal flora in the throat. Xantocid is composed of Xantocillin and Thyrotricin in the form of a lozenge, one administered every hour or every 2 hours. Our case material consisted of 38 children between the ages of 3–15 years who had been admitted to the scarlet fever ward. Bacterial cultures for streptococci as well as general flora were made from throat swabs taken on admission and every morning. Four to seven lozenges were given for 3–5 days.

Of 2? patients with uncomplicated scarlatina, 1 had hemolytic streptococci (+ +) on admission while 1 had streptococci (–) after Xantocid therapy. Of 12 patients with scarlatina and impetigo or with lymphadenitis, 10 had streptococci (+) on admission and 9 after therapy. One patient became bacteria free but pharyngitis remained and he was subfebrile. Of 4 patients with the diagnosis desquamation of scarlet fever, scarlatina or streptococcangina, 2 had hemolytic streptococci (+ +) on admission and 4 (+ +) after Xantocid therapy. The chances of finding β-hemolytic streptococci in the throat are greater in the morning, so that any substantial increase of the bacterial flora is not likely.

Of all 38 patients, 13 (one-third) had signs of infection remaining after Xantocid therapy. One developed otitis media and another streptococcal vulvovaginitis during this therapy.

This investigation shows that Xantocid does not appear to affect an abundant streptococcal flora in the throat. One cannot expect it to be an effective weapon against the spread of β-hemolytic streptococci in, for example, school or closed institutions, as the manufacturer presumes. It is true that a scarlet fever ward is an environment rich in bacteria, but so is the classroom and the family milieu.

B. Urnng Immune-electrophoresis of the spinal fluid in cases of infections of the central nervous system and of polyradiculitis

By means of immune-electrophoresis, the spinal fluid has been studied from 19 cases of parotitis meningitis, as well as 17 cases of viral meningitis of unestablished etiology 4 cases of bacterial meningitis and 5 cases of polyradiculitis. This report concerns the occurrence of pronounced α_2 macroglobulin, β_1 lipoprotein, fibrinogen, transferrin of the serum type β_2M and β_2A-globulin, as an expression of pathological protein patterns.

Two cases of parotitis meningitis and 4 of the cases of viral meningitis had normal spinal fluids. N case of bacterial meningit or polyradiculitis had normal spinal proteins. Instead these showed the most pathological picture of all CNS diseases. β_2A-globulin was the commonest pathological fraction in parotitis meningitis and β_1-lipoprotein in the other viral meningitides. In bacterial meningitis and polyradiculitis, nearly all the abov -named substances were present F bri nogen could be demonstrated in the spinal fluid when the protein level was 27 mg . Transferrin of the serum type was unquestionably commonest in bacterial meningitis and polyradiculitis. The spinal fluid was most pathological at the peak f the disease after which relatively parallel restitution occurred. The pathologic fractions are not thought t be an important in regard t the development of post encephalitic symptoms. In bacterial meningitis and polyradiculitis, the spinal fluid changes sometimes persist fter the patient has recovered.

B. Thelme I Jungner and *B Åberg* The use of the ultramicromethod in a linical chemical laboratory

The analysis of hloride calcium, phosphorus, urea and total proteins in blood serum has been carried out using Beckman Spinco modification of the ltra-microtechnique *I Rane*. The colorimetric method cannot at present be entirel ev luated the manufacturer has not been able t deli er the microtizer part of the equipment. The titration methods give satisfactory accuracy for practical linical work, but are not as precise as the corresponding macromethods. The authors would like to stress that it is necessary that the personnel who work with this technique be extremely well trained. The method greatest advantages are that it saves space and glassware and that material consumption is, bove ll, especially low Therefore this method ought to have definite place in pediatric practice as well as in work with experimental animals, rats, mice and so forth

A. Espmark and *E. Rabo* Smallpox vaccination during the first trimester of life Percentage of positi reactions in relation to vaccine strength

In *Svenska Läkartid* 56 82 1959 Rabo has given an account of the percentage of posit reactions and advantages of vaccination during the first months of life In new investigation more than 300 children ha been vaccinated, partly with undil ted accine and partly with vaccine dilution down t 1/316. The takes with different accine titers vary from to 100%, and ppear to follow normal distribution curve A group of children, 5–12 months of age has been vaccinated with the same vaccine dilutions. In order to obtain the same percentage of positive reactions in both groups, one must use a vaccine ten times stronger for the younger children. This investigation has shown that one may obtain the desired percentage of takes" even low t 100 . by hoosing the appropriat vaccine titer

A. Espmark and *E. Rabo* Antibod formation following smallpox accination before the ge of 3 day

Tw groups of hildren, the first one month of age and the second 9–12 months old were in estigated with regard to the presence of neutralizing antibodies before and fter smallpox accination. Twenty-six ch ldren in each ge group ha e until now been examined serologically The titration

of antibodies was done by a screening method, whereby filter paper discs with serial dilutions of serum were placed directly in virus-infected tissue cultures. Localized viral growth inhibition was interpreted as neutralization.

Of the 6 younger infants, 23 had antibodies in the prevaccination serum. In the older group, antibodies were found in two of 26. In sera taken 1 month after vaccination, the titer values obtained ranged from 1 10 to 1 100 with a distribution which suggested a slightly lower average value for the younger group. The further development of these titers will be estimated by repeated titration of sera taken one year after vaccination. Until now 9 months sera have been taken from five children in each age group and titrated simultaneously with the serum samples from the same infants obtained earlier No obvious difference in the 9 month titers f the two groups was demonstrated. The number f samples is, however too small for minor differences to be verified. One month after vaccination, six of the ten infants had titers 1 100 and four had titers 1:3.. In the 9 month sera, four of the higher titers had decreased three-fold, while the lower titers remained unchanged.

The question as to whether the lower antibody formation demonstrated f the 1 month old infants depends on the maternal antibodies or on some incompleteness in the immunological maturity cannot as yet be answered by our results. In any event, the difference demonstrated is small, and it may be assumed that even in the younger infants, the first vaccination carries out its most important function, that is t produce the sensitization to vaccinia virus which conditions a good booster response to revaccination.

Meeting Feb 10 1961

B Persson and *G Sterky* A case of non-hemolytic hyper-bilirubinemia

A 13 year old girl with persistent hyperbilirubinemia since birth is described. She had no family history of jaundice and was asymptomatic except for iron deficiency anemia t the time of her last examination. The level of bilirubin through the years varied between 3.6 and 10.0 mg% with negative direct and always strongly positive in direct Hijman van den Berghs reaction. It could be shown (eg normal COHG and Cr^{51} survival) that no hemolytic process was involved. Liver function tests, liver histology and cholecystography were normal. The case was diagnosed as Gilbert disease The authors discuss the progress made during the last few years in understanding defect in bilirubin metabolism The difficulty of drawing conclusion from the results of loading with salicylat is underlined and the findings t chromatography of bile acids discussed In this patient a very low level of chenodeoxycholic acid was found which may suggest the presence f other enzymatic defects in the liver cells. The existence of hitherto unknown pathways of bilirubin metabolism are considered.

A Engström and *B Persson* Hypoproteinemia with edema and albumin loss in the gastro-intestinal tract

A preliminary report is given about an infant with polycystic kidneys, intestinal edema and hypoproteinemia who has been under observation since 4½ months of age. Serum protein levels varied bet een 2.5-3 6 g%. Laboratory examination of blood and urine were normal. Liver pancreas and adrenal function as well intestinal absorption of fat and carbohydrates are normal. Studies with I^{131} albumin i.v revealed a loss f lbumin into the gastrointestinal lumen estimated to be about 4 g per da Treatment with high doses f albumin i. had only a transient ffect Triamcinolone was of no value A repeated I^{131}-albumin study during a trial course of human growth hormone showed no change albumin loss, but suggested increased protein synthesis.

Hypoproteinemia in hildren is discussed. The albumin studies were performed together with a group t the Gustaf V Forskningsinstitut Stockholm. The growth hormone was kindly put at our disposal by Prof. Gemzell, Uppsala.

Yngve Larsson, Göran Sterky Kristina Ekengren, and Tage Möller Physical fitness and the influence of training in diabetic adolescent girls

The physical working capacity (PWC) was studied in a group f 22 adolescent diabetic girls and compared with that of a control group of 47 non-diabetics of the same sex and age The PWC w slightly lower in the diabetic group, the difference being most evident in the age-group of 15–18 years. In the diabetic group, the relations between PWC and body weight, age and heart volume were the same as among on diabetics. There was a regular decrease of blood sugar during work and a highly significant correlation between the initial blood sugar value and the extent of blood sugar decrease After training in moderate long term as well in a strenuous short-term program, the PWC increased in the majority of the diabetic patients. It was most marked in the girls of 13–14 years who took part in hard training. pronounced improvement being noticed also in he diabetic state in spite of unchanged insulin dosage and a 30 larger calorie suppl There was significant rise of blood cholesterol during the work tests. Also the levels of cholesterol and triglycerides in the blood but not of phospholipids) were significan ly increased, when determined a few days after he severe training program.

The value of exercise and raining in the treatment of juvenile diabetes emphasized the fa ourable ffect being especiall conspicuous in patients with high bl d sugar level. During periods of intensified p ysical activity the caloric suppl sh uld be in creased, rather than he insulin dosage decreased.

C. G. Bergstrand and M Otto Attempted suicide in hildren and adolescents

The incidence of attempted suicide in persons 21 years of age or less was thought to be of interest. Four hundred and seventy one hospitals in the entire country were re quested to send in the appropriat records during the 5-year period 1955–1959 A total of 1727 cases (351 boys (20.3%) and 1376 girls (79.9%)) was collected from 458 institutions (97.3%) The youngest case was a 10½ year old boy The incidence of suicide ttempt increased with age and was greatest at puberty An absolute increase in incidence wa also noted during the 5 year period of study Seasonal variation was also noted. The smallest number of cases occurred in June and Jul (92 and 81 cases respectively) and the largest umber in November (147 cases). Thirty-eight per cent of the cases came from Stockholm, Gothenburg and Malmö, the inhabitants of which account for one-fifth of the total population of Sweden. The majority (8_5%) were from the lower class and 4 6 % were foreigners (78 cases) Alcoholism was found in 14.8% and psychiatric disease in 27 6% of the parents. Forty three per cent of the cases came from broken homes. Psychiatric disease was present in 19 of the boys and 8% of the girls. The method of suicide used by boys tended to be more active (hanging, shooting) while girls tended to take overdoses of tablets, etc Twenty-nine per cent had ttended psy chiatric clinic or been in mental hospitals while 70.4 had been treated for somatic disease Psychiatric consultation was obtained in 69 4 A need for continued psychiatric care was found in 31 % of the boys and 19.3% of the girls. Fifteen per cent of the persons had previously attempted suicide and this was more common amongst the boys.

K M Herrlin and P O Hillborg Cerebral symptoms in the juvenile form Gaucher disease

Six familial cases of Gaucher disease are described. The patients are 6–20 years old and are all alive Diagnosis has been con-

firmed in two by chemical analysis of the spleen. Splenectomy was performed at 1½–12 years. Within 3 years of the operation, skeletal changes occurred in all cases. Cerebral symptoms have gradually also appeared in all after operation. Five are mentally retarded. Muscular incoordination primarily affecting the eyes is present in 5, while 2 also have trismus. Three have epilepsy. All of the patients have abnormal electroencephalograms, 3 of which show significant changes with spikes, and sharp and slow waves with shifting localization. The cerebral symptoms are similar to those found in the infantile form in which they are attributed to deposit of cerebrosides in the reticuloendothelial cells of the brain vessels. The pathogenesis in the juvenile form is probably the same. It is possible that the cerebral symptoms as well as the early appearance of the skeletal changes have been precipitated by splenectomy. (Will be published in *Acta Paediat.*)

C. G. Bergstrand, Birgit Czar and P. H. Tarukoski. Serum haptoglobin in infancy.

Haptoglobin is defined as group of mucoproteins with the electrophoretic properties of α_2-globulins. Haptoglobin forms with hemoglobin a very stable complex with peroxidase activity which may according to Jayle, be used for quantitative determination of the serum haptoglobin content. With Jayle's activation method, the haptoglobin level in sera from fetuses, newborns and infants was determined. In fetal and cord blood, no haptoglobin was demonstrated. During the neonatal period (full term and premature infants) small amounts of haptoglobin were found only in a few cases. After the newborn period, the haptoglobin level tends to rise gradually and after 3–4 months of age values below 30 mg per 100 ml serum, i.e. the lower limit for normal adults, are rarely demonstrated. Fetal hemoglobin bound to haptoglobin seems to have a peroxidase activity which is higher than the corresponding complex of adult hemoglobin. Preliminary experiments suggest that haptoglobin injected intravenously into newborn infants is rapidly eliminated.

ANNOUNCEMENT

The X International Congress of Pediatrics

The X International Congress of Pediatrics will be held in Lisbon, Portugal Sept. 9 to 15, 1962 with Professor C. Salazar de Souza as President and Professor Mário Cordeiro as General Secretary. There will be three plenary sessions, which will take up three mornings with the following items: 1. Problems of the newborn infant. 2. Pediatric problems of tropical climates and countries in development.—3. Pediatric doctrine. The remaining scientific programme is divided into 15 sections, for each of which subjects of more topical importance has been chosen. An extensive social program is planned as are scientific exhibits, industrial exhibits, and exhibitions of art and children literature. The address of the Secretariat: Av. 28 de Maio, Lisbon, Portugal.

ANNOUNCEMENTS

Congresses

The II International Congress of Mental Retardation

Vienna, August 14–19 1961 President: Professor K. Kundratitz, Pediatric Clinic, Lazarettgasse 14, Vienna, Austria. The principal subjects t be discussed are: 1 Organic causes of mental retardation.—2. Endocrine disorders and mental retardation. —3. Encephalopathy and mental retardation.—4. Genetic and metabolic problems. —5. Mental retardation caused by psychoses in childhood.—6. Pseudo-feeblemindedness. —7 Diagnostics and tests.—8. Evaluation of histories.—9 Social adjustment of mentally retarded children.—10 Child guidance. —11 The physician and special schools.—12. Pharmacotherapy

The Annual Congress of the Pediatri Society f North Western Germany

Lübeck, June 2–4 1961 President Professor J Jochums, Kinderklinik, Kronsforder Allee 71/73 Lubeck, Germany There will be refresher courses in Orthopedics and in Dermatology The scientific subjects to be discussed are: 1 Constitution and 2. Psychotherapy

The 60th Congress of the German Pediatric Society

Heidelberg Sept. 11–13, 1961. President Professor Ph. Bamberger Universitäts-Kinderklinik, Heidelberg, Germany The principal subjects are: 1 Convulsions in childhood.— Inborn errors of metabolism. —3. Malignant tumors in hildren. Information may be obtained from the General Secretary Professor K. Schreier Universitats-Kinderklinik, Heidelberg

The XIII Congress f the Nordi Pedi tric Association

Copenhagen, June 25–29 1961 President Dr med. P W Braestrup, General secretary Dr med. E. Winge Flensborg Principal subjects to be discussed. 1 The influence of prenatal and natal factors for development and for diseases; prespective and longitu dinal studies.— 2. Epilepsy in childhood.—3 Steroid treatment of acut lif -endangering infections.—4. Prognosis of asthma and asthmatic bronchitis in children. Address f the Secretariat, Amtssygehuset, Hellerup Denmark.

BOOK REVIEWS

André-Thomas, Y es Chesni and S Sai t Anne Dargassies The Neurological Examination of the Infant.

National Spastics Society London, 1960. 50 pages. Price 5/ net or $1 —

This is the first number of Little Club Clinics in Developmental Medicine published as Supplements t the excellent Cerebral Palsy Bulletin It gives an account of some guide lines of neurological examination and some typical responses f the normal newborn and older infants. The aim is to make topographical diagnosis and to describe the neurological mechanisms implicated. The editors of the booklet, R. C MacKeith, P E. Polani and E. Clayton Jones, give introductory information concerning the type of examination used by André-Thomas as well as an interpretation of the findings. The text is richly illustrated by drawings from photographs and ciné films. This is useful booklet for pediatricians and students.

L. Emmett Holt, Jr., P. György, E. L. Pratt, S. E. Snyderman and W. M. Wallace: Protein and Amino Acid Requirements in Early Life.

New York University Press, New York, 1960. 63 pages. Price $1.00.

In this pamphlet present knowledge of the protein and amino acid requirements in early life are summarized. The following items are discussed: protein intake and body composition, evaluation of protein requirements and of protein quality, amino acid requirements and amino acid balance, desirable intake of protein and amino acids and the need for further research. It is not possible to go into details. Be it sufficient to say that the responsible authors, who are well known experts in pediatric nutrition, have critically evaluated the current literature. About 200 references.

E. Gedda: The Significance of the Premature Child's Low Birth Weight for its Vitality and Social Prognosis.

Almqvist & Wiksell, Uppsala 1960. Thesis, University of Gothenburg.

This work has special value as a parallel and complement to a study made by Ingvar Alm entitled "The Long term Prognosis of Prematurely Born Children" (*Acta Paediat.*, Suppl. 94, 1953). Like Alm's work it is based on material culled from maternity hospitals, well baby clinics, school health services and official social registers, such as that of the Royal Temperance Board and Central Register of Criminology. Social status and adaptation as well as income were also studied. Alm's material consisted of 999 premature and 1002 full term babies (as controls) born 1901–1911 in three maternity hospitals in Stockholm. The present material consists of 742 prematures and 426 controls born 1930–1936 in the maternity hospital of Gothenburg. Alm's material was selected, and only included boys legitimately born. Gedda's material comprises all prematures, girls as well as boys, regardless of legitimacy and also stillborns of the same birth weight. Gedda's material is probably more typical of the population of Gothenburg than Alm's was of Stockholm, as the percentage of institution deliveries was higher in Gothenburg in the thirties (82–85%) than in Stockholm during the early decades of this century (50–75%).

Gedda has followed the same principles as Alm when subgrouping the material by dividing the children into single and multiple births. The tables are arranged in the same way as Alm's, which makes comparison easy.

Factors which may influence birth weight are considered. As this material is based on obstetrical case histories which are 25–30 years old, the results are somewhat difficult to evaluate. Gedda then examines the mortality rate at different ages and has been able to follow nearly all living patients to the age of twenty. The following results were the most important.

During the period 1930–1936, prematures constituted about 5% of all live births in Gothenburg. Their mortality during the first week of life was 19.1% as compared with 0.7% for children born at term and their perinatal mortality 30.2% as compared with 2.1% for controls. The infant mortality minus the mortality during the first week was 7.2% for prematures compared with 1.1% for controls. During the second year of life the mortality was 3.1% for prematures and 2.4% for controls. After five years of age no difference was found in the mortality risk between the two groups. The difference in morbidity declined similarly and was not present at school age, apart from that associated with sequelae from birth injuries, which are probably more common among prematures. Prematurely born children were considered by physicians as well as by their parents as "fragile" more often than were the controls. This may be due more to their attitude than to prematurity per se. It was shown that premature children on the average were somewhat smaller even at school age. Regarding psychic develop-

ment the investigation could not verify any significant differences between prematures and controls, but here a more detailed study seems indicated. Like Alm, the author found no difference between prematures and controls regarding social capacity working capacity and social adaptation. Though the author's figures indicat an increase frequency of prematures amongst retarded and other minus variants in school-age he has furnished evidence that the premature child handicap has been principally overcome by the age of one year

*P Végh*e*lyi* Die Künstliche Hibernation. Hungarian Academy of Sciences. Budapest. 1960. 612 pages, 107 figures. Price not given

The well-known Hungarian pediatrician, Véghelyi, has written this important monograph on the theoretical problems and practical experiences of artificial hypothermia both chemically and physically induced. Every aspect of the problem is considered. The indication, technique and clinical experience in various fields of medicine as well as the contra-indications and side effects of hibernation are described in detail in separat chapters. For pediatrician the experiences of induced hypothermia in the treatment of perinatal disorders, infantile diarrhoea, infectious diseases and Waterhouse-Friderichsen syndrome are of special interest. Due consideration is paid to the publications and experiences of other investigators and the tabulated references number 1568

F G. Young (Editor) et al Insulin. British Medical Bulletin. Volum 16, Number 3, September 1960, 167–264 Price 20 Sh

This publication represent most valable and competent review on insulin by leading Canadian and British uthorities. Fort years have passed since the discovery of the hormone and the act ties of the pioneer Toront institut during this vent ful period of medical history are described by Charles H. Best in the introductory paper Next, F Sanger in an article on the chemistry of insulin shows how through the use of advanced chemical technique he succeeded in finding the double-chained structure of the molecule The problems of insulin assay both *vitro* and *in vivo* are analysed in several papers and comparison made between the often remarkably divergent results obtained with different methods. Insulin antagonists and insulin antibodies are also reviewed and as regards the labile type of juvenile diabetes the hypothesis is given (Vallance-Owen) that fluctuating overaction of the insulin-antagonistic pituitary-adrenal system may be a cause of the "brittleness and that treat ment with adrenal steroids in doses suffi cient to suppress the endogenous production of these hormones might stabilize the disease The mechanism of action of insulin is a field of some controversy illustrated in several contributions. Some investigators believe that the manifold actions of insulin with effects on carbohydrate (glycolysis), fat (lipogenesis) and protein (growth) metabolism are explicable in terms of a single effect, probably located to the processes governing the entry of glucose into the cell. Others believe that insulin acts on different intracellular metabolic pathways or enzymatic (hexokinase) reactions. "Nevertheless, the glimmerings of a reasoned unity are beginning to be discernible in the fog of uncertainty (Randle & Young). The last part of the volume has a more clinical aspect, discussing indications and contraindications for the use of different insulin preparations, treatment of ketosis, reactions, etc. Finally two articles describe the hemistry and linical use of hypoglycaemic substances other than insulin, the sulphanilyl as well as the guanidine derivatives. In juvenile diabetes the effect of the former is only temporary during an early phase of the disease but in adults, too the incidence of secondary failures is increasing As regards the guanidine preparations their mode of action as well as indications for use are still unsettled. Unt l more is known it seems wise to use them only in carefully conducted clinical trials.

Yngve A A Larsson, Stockholm

TABLE 1 *Medical schools in S.-E Asia.*

Country	Duration of medical course in years	Internship	Degree	Medium of instruction	Tuition fees	Service to government
Afghanistan	6	Compulsory	Dr med.	Farsi	Nil	Permanent
Burma	5[a]	Not available	M.B B.S.	English	$3 per month	Nil
Ceylon	5[b]	Available not strictly enforced	M.B., B.S.	English	Nil	Nil
India	5[c]	Compulsory where available	M.B., B.S.	English	Average $50 per year	Nil
Indonesia	7[a]	Part of the curriculum 2 years	Dr med.	Indonesian	$5 per year	Three years after graduation
Portuguese India	5½	6 months compulsory	Médico-Cirurgião	Portuguese	Nil	Nil
Thailand	4	Available, but not compulsory	M.B	Thai (with technical terms in English)	In stage of revision	One year for each year of subsidized studies

a Plus two years pre medical training
b Plus one year preparatory course.
c Being reduced now to six years.

There is no internship year The qualification is M.B (Bachelor of Medicine) B.S. (Bachelor of Surgery)

There are no tuition fees in *Ceylon* either but students who fail in examinations and have to repeat a course, pay the equivalent of $ 35 per semester Full registration is granted after one year's internship in an approved hospital. Provisional registration is permitted immediately after graduation. The qualification is M.B B.S.

In *India* annual tuition fees range from the equivalent of $25 (in six institutions) to $100 (in one only) the average being $50 Recently an internship year has been introduced in some or most of the medical colleges which is now in the process of being reduced to six months. There is no definite provision for part of the internship service to be spent in paediatrics. The qualification is M.B B.S.

In *Indonesia*, the annual tuition fees are 40 rupiahs (about $5 at the present official rate of exchange). There is no internship but during the sixth year of studies the student does practical work in the paediatric department. Graduates must serve the government for three years. The qualification is Dr med.

In *Portuguese India* there are no tuition fees After graduation there is a six-month compulsory internship Paediatrics for this purpose is part of the obstetric training The qualification is "Médico-Cirurgião" and is not recognized outside the colony

In *Thailand*, there were no tuition fees till recently Now students can only be exempted if they undertake to serve the

TABLE 2. *Students in paediatrics in S. E. Asia.*

Country	Total number of medical teaching institutions	Medical schools teaching pediatrics: No. of institutions	No. of students	No. of students admitted 1959: M	No. of students admitted 1959: F	No. appeared for examination 1959	No. passed	% failure
Afghanistan	1	1	255	83	37	33	33	0
Burma	2	2	1,341	229	100	231	104	55
Ceylon	1	1	699	106	27	154	123	20
India[a]	62	36	18,617	2371	967	4079	2603	46
Indonesia[b]	6	4	4,068[c]	488	73	253	205	19
Portuguese India	1	1	100	23	XII	Unknown	23	Unknown
Thailand	2	2	1,601	167	53	197	163	18

a Only 33 of the medical colleges teaching paediatrics graduated students in 1959.
b Only three of the medical faculties teaching paediatrics graduated students in 1959.
c This number includes students of dentistry in Jogjakarta.

government after graduation. Internship is not compulsory. The qualification is M.B.

The medium of instruction in *Burma*, *Ceylon* and *India* is English. In *Afghanistan*, national teachers teach in Farsi, and lectures of foreign teachers are being translated. In *Indonesia* the medium of instruction is Indonesian but guest teachers often use their own languages. In *Thailand* while Thai is used, technical terms are in English. In *Portuguese India* the medium of instruction is Portuguese.

3. Distribution of Schools and Students in Paediatrics

In one country of the region—Nepal—there is no medical school yet. In *Afghanistan*, the present output of graduates is considered sufficient for the time being. In *Burma* the recently established second medical college in Mandalay is part of the University of Rangoon. In *Ceylon* plans for the establishment of a second medical college in Kandy are nearing completion. In *India* 14 new medical colleges were established during the last four years making a total of 6.. Only two of those have reached the stage of clinical teaching: four other medical colleges have not yet fully developed the clinical curriculum. Thus, paediatrics is taught at present in 36 out of the existing 6 institutions. In *Indonesia*, two medical faculties were established since 1955 making a total of six. Paediatrics is not taught yet in the new institutions. The "medical surgical" school in Goa (Portuguese India) is without full university status. In *Thailand* in addition to the two medical schools in Bangkok, a third is under preparation in Chiengmai.

4. Paediatrics as a Speciality

In *Afghanistan* where the faculty of medicine was established in 193.., there was no paediatric teaching till 1943. At present paediatrics is a full-fledged de-

TABLE 3 a. *Departments of paediatrics independent and dependent.*

Country	No. of institutions teaching paediatrics	Fully independent paediatric department	Not fully independent paediatric department
Afghanistan	1	1	—
Burma	1	—	1
Ceylon	1	1	—
India	36	24	10
Indonesia	4	1	3
Portuguese India	1	—	1
Thailand	2	2	—

partment, and is a "major subject" in the final examinations.

In *Burma* the paediatric wards and out patients services are under a paediatrician, but administratively paediatrics is part of the department of medicine and does not constitute a separate subject in the final examination.

In *Ceylon* where the medical college was established in 1870 a chair of paediatrics has existed since 1949 At the final examinations paediatrics is included in medicine.

In *India* all stages of transition from a "subspeciality of medicine to a separate chair are found. However in no institution is paediatrics considered a 'major subject, nor is it a subject for the final examinations; usually one or two questions in the medical examination paper deal with paediatric problems.

In *Indonesia* the paediatric department of the faculty of medicine of Djakarta is a large unit under a full professor Paediatrics is considered there a major subject and a special examination is held. In the other medical faculties the paediatric units are small, and teaching is limited, administratively they are under the department of medicine.

In *Portuguese India* paediatrics is not taught as a separate subject

TABLE 3 b. *Paediatric departments in India. development during the last four years.*

Total number of medical colleges teaching paediatrics		Independent departments: In charge of full professors of paediatrics		Independent departments: In charge of an associate professor a reader lecturer or an honorary paediatrician		Under the professor of medicine: Paediatric department supervised by professor of medicine		Under the professor of medicine: Paediatric wards actually part of the medicine department, and all teaching done by the professor of medicine	
1955	1959	1955	1959	1955	1959	1955	1959	1955	1959
31	36		11	9	14	11	6	9	5

In *Thailand*, paediatrics was separated from medicine in 1945 It is not yet considered a major' subject.

5 Teachers in Paediatrics

Some of the paediatric teachers are full-time some are part-time and some are honorary With very few exceptions, however the time devoted to teaching is independent of the status of the teacher For the purpose of a general review it may be accepted that the actual time the chief of the department devotes to teaching is 1 2 hours a day in all, or most institutions.

At Kabul medical faculty—*Afghanistan*—there is one professor two associate professors, one reader and one lecturer The ratio of teachers to students is one to seven.

In *Burma* the teacher–student ratio is the lowest in the region, there being only one part time lecturer for 240 students in paediatrics.

In *Ceylon* there is one professor and one lecturer but most of the teaching is done by junior assistants, after two years of internship

In *India*, there are considerable differences The highest number of paediatric teachers— in two institutions only—is six; the ratio of teachers to students in these two units is 1 [illegible]0 and 1 35 respectively The lowest ratio is 1 305 In five colleges there are as yet no special teachers in paediatrics and the subject is taught by the professors of medicine (see Table 3b)

In Djakarta, *Indonesia* there is the largest number of paediatric teachers of any institution in the region—18 Fourteen of these are classified as lecturers. However the teacher–student ratio is only 1 9 lower than in Kabul. In the other medical faculties in that country there are very few paediatric teachers.

TABLE 4 *Teacher–student ratio*

Country	Average	Range
Afghanistan	1 7	—
Burma	1 40	—
Ceylon	1 45	—
India	1 70	1 300–1 [illegible]0
Indonesia	1 16	1 100–1 9
Thailand	1 13	—

In *Portuguese India* there are no separate teachers in paediatrics

In *Thailand*, the teacher–student ratio is next only to Afghanistan, in this region.

6 Teaching Facilities

A. *Hospital Beds*

Traditionally hospital beds are often considered the most important, sometimes the only necessary facility for teaching of paediatrics. This is, of course even less correct in this part of the world than in economically more advanced countries. Only very sick children can be admitted to paediatric departments of teaching hospitals here, and the student, if this is his only or main, training field, sees chiefly the final stages of fatal conditions.

But even so, the numbers of beds in teaching hospitals are very small, and some of the so-called "paediatric beds" are for surgical" conditions in children—actually under the supervision of th surgeons. Occasionally the paediatricians may be called upon to advise on the general care of children hospitalized for surgical conditions but as a rule the surgical

TABLE 5 *a* *Beds for children in teaching hospitals.*

Country	No. of institutions teaching paediatrics	Number of "medical" beds for children			No. of "surgical" beds for children		
		Infectious included	Infectious excluded	Separate Infectious	Under surgeons	Under paediatric surgeons	Under paediatricians
Afghanistan	1	40	—	—	—	—	—
Burma	2	80	—	6	30	—	—
Ceylon	1	—	250	24	106	—	—
India	36	566	1096	94	635	65	10
Indonesia	4	180	65	20	—	—	—
Portuguese India	1	—	—	—	—	—	—
Thailand	2	135	60	80	13	—	—

paediatric beds are unrelated to paediatric teaching activities. In one unit only in India, the surgical beds are under the care of the paediatrician, and in two other also in India, under paediatric surgeons.

As a rule, 'medical and 'infectious beds are available for paediatric teaching purposes, but not necessarily all beds. Some institutions have special wards earmarked for teaching and there is, in that case administrative divison between the service and the teaching unit.

B. *Out patient Services*

Separate daily out-patient clinics for children are held in all but three of the teaching hospitals in which paediatrics is taught. The average number of child out-patients per day ranges from 140 in Indonesia to 1°00 in Ceylon. About 50 % are, as a rule, new cases. Very few if any nurses are available to assist the doctors.

Some of the paediatric teachers select a limited number of child out patients for teaching, or have special sessions to which selected cases are invited. Although there appears to be no third alternative, these methods are unsatisfactory in as much as teaching is not done under normal conditions of service

TABLE 5 *b* *Bed–student ratio*

Country	Average	Range
Afghanistan	1 1	—
Burma	1 3	—
Ceylon	1 2	—
India	1 3	1 18–1 1
Indonesia	2 1	1 2 –3 1
Thailand	3 2	—

C. *Preventive Child Care*

Only five teaching institutions, all in *India*, take advantage of existing maternal and child health centres or child welfare centres for teaching of preventive child care Preventive service units for children are available in all cities or towns where medical schools are located, but for various reasons those are not used for teaching These reasons may be summarized as follows:

1 Insufficient awareness on the part of

TABLE 6. *Facilities for out-patient teaching*

Country	No. of institutions teaching paediatrics	Out-patient services available for teaching	Average number of out patients per day	
			Total	New
Afghanistan	1	1	200	60
Burma	2	2	230	110
Ceylon	1	1	1200	600
India	26	25	170	70
Indonesia	4	3	140	60
Portuguese India	1	—	—	—
Thailand	2	2	145	50

teachers of the importance of preventive paediatrics.

2. No teaching time allotted by the faculty for this purpose.

3. Difficulties arising out of the fact that the preventive service units are under a different authority from the teaching institution.

4. Some public health officials in charge of preventive services do not welcome teaching paediatricians to use their institutions.

5. Some teaching paediatricians prefer to have their own child welfare clinics rather than use existing ones.

In *India* a number of teaching institutions now have departments of preventive and social medicine. Some of the professors of preventive and social medicine consider preventive paediatrics to be their domain. Where the teaching paediatricians are not too keen on preventive aspects, they welcome the initiative of the department of preventive and social medicine to take it on. In a few instances, the two professors co-operate in the teaching of preventive paediatrics, and again, in some others, the professor of preventive and social medicine is quite ready to give up some of the hours allotted to him to the paediatrician, for him to use it for teaching preventive paediatrics.

TABLE 7. *Facilities for teaching preventive paediatrics*

Country	No. of institutions teaching paediatrics	Child welfare clinics available for teaching purposes	
		Attached to teaching hospital	Under different authority
Afghanistan	1	—	—
Burma	2	—	—
Ceylon	1	1	—
India	26	11	8
Indonesia	4	1	—
Portuguese India	1	—	—
Thailand	2	2	—

TABLE 8 "*Rooming-in*"

Country	Total number of teaching institutions with obstetric services	Infants with mothers	Separate nurseries
Afghanistan	1	1	—
Burma	2	—	2
Ceylon	1	1	—
India	35	20	5
Indonesia	4	—	4
Thailand		—	2

whoever looks after the mother is also responsible for the infant.

"Rooming in" is not an accepted principle in South East Asia and, so far no institution which had a separate nursery for newborn has changed back to "rooming in" On the other hand recent attitudes in western countries may have influenced institutions, at least in India not to establish separate nurseries where such did not already exist.

D *Newborn Care*

Newborn care is taught by obstetricians, paediatricians, or not at all. While some obstetricians consider newborns to be their domain both in respect of teaching and service others are only too glad to hand over supervision to the paediatrician.

The paediatricians, on the other hand are often reluctant to assume responsibility for newborns because of lack of time

An important reason for leaving care of, and teaching on, newborns to the obstetrician in some countries of this region is the prevalent system of "rooming in" Where infants are with their mothers it is simpler and appears more sensible that

E. *Special Clinics*

There are a number of special clinics in India attached to some institutions, which are sometimes but not invariably used for undergraduate teaching None in other countries, so far

7 Hours and Methods of Instruction

Paediatrics is taught in the third, fourth, or fifth year during one two or three years. Experimentally some aspects of preventive paediatrics are taught in two institutions in India also in the pre-clinical period, in co-operation with the department of preventive and social medicine

TABLE 9 *Newborn care.*

Country	Total no. of teaching institutions with obstetric services	Newborns under paediatrician	Newborns under obstetrician			Premature unit	
			Paediatrician consulted regularly	Paediatrician consulted occasionally	Paediatrician consulted exceptionally	Under paediatrician	Under obstetrician
Afghanistan	1	—	1	—	—	—	—
Burma	2	—	—	—	2	—	—
Ceylon	1	1	—	—	—	1	—
India	33	4	7	20	4	3	4
Indonesia	3	1		—	—	1	—
Thailand			—	—	—		—

TABLE 10 *Special d nics*

Child guidance	Handi capped children	Neuro-logical	Nutrition	Spastics	Liver con ditions	Tuber culosis	Mental deficiency	Rheumatic heart disease
9	5	3	2	3	1	3	1	1

Didactic lectures range from a few in some institutions in India to 230 in Afghanistan.

Bedside teaching is practised in all countries, but in some instances this is actually didactic lectures delivered at the bedside, instead of in the lecture hall Where sufficient junior tutors are avail able bedside teaching is practised more efficiently

Not much time is devoted to out patients and child welfare teaching for reasons already stated (see Section 5, paras. B and C).

There is no clerkship in Afghanistan Burma, Ceylon Portuguese India or in six of the teaching institutions in India. Clerkship from 2 to 107 weeks is available in all but six institutions in India and in Indonesia and Thailand. Not infrequently clerkship means little more than writing case sheets for the doctors.

Home visiting by students of paediat rics has recently been introduced in Colombo In some of the Indian institutions home visiting is an important part of the activities of the department of preventive and social medicine and some of it is concerned with child care. This is espe cially so where the teachers of paediatrics and preventive and social medicine co-operate.

A major opportunity to impart tech niques of health education is given in a large number of teaching institutions in India and Kabul through the presence of mothers on the wards. So far little advantage is taken of this opportunity although more

TABLE 11 *Hours of teaching*

Country	Year(s)	No. of didactic lectures		Hours of bed-side teaching		Hours of out patient teach ing		Preven tive child care	No. of weeks of clerkship	
		Average	Range	Average	Range	Average	Range		Average	Range
Afghanistan	IV V	230	—	85	—	83	—	XII	Nil	—
Burma	V	20	—	12	—	12	—	—	4	—
Ceylon	III, IV	46	—	132	—	16	—	Yes	10	—
India	Variable	30	4–75	53	0–156	36	0–48	In 10 only	8	0–8
Indonesia	IV V	105	12–200	43	23–70	37	12–60	Yes	7	2 14
Thailand	III IV	75	73–77	75	60–90	42	4–80	In one school only	56	6–107

TABLE 1? *Attitude to mothers of hospitalized children.*

Country	Total no. of teaching institutions	Banned	On sufferance	Welcome
Afghanistan	1	—	—	1
Burma	2		—	—
Ceylon	1	1	—	—
India	36	4	14	18
Indonesia	4	4	—	—

and more institutions begin to realize the great possibilities of educating mothers during their stay in hospitals; this also permits medical students to study mother–child relationships and techniques of health education. The best facilities for mothers exist in Kabul where they are given beds next to their children and food. In India, attitudes to the presence of mothers differ and facilities for their accommodation are limited

8. Trends and Outlook for the Future

The importance of teaching paediatrics as a separate discipline is growing in all countries of the region. Serious efforts are being made in this direction.

At the same time, more emphasis is being laid on teaching of promotional and preventive aspects of child care and the use of peripheral services for instruction of medical students.

References

1 American Academy of Pediatrics: Child Health Services and Pediatric Education. New York Commonwealth Fund 1949
2. Canadian Society for the Study of Diseases of Children: Survey Canadian Medical Schools 1947–48 Toronto, privat ly printed, June 1949
3. VEENEKLAAS, G M. H.: Paediatric education in Europe. *Hel Paediat Acta 12* Suppl. 7 1957
4 WEGMAN M. E., HUGHES, J G and POTTER, R. R.: Pediatric education in Latin America. *Pediatrics, 22* 203, 1958.
5. ROBINSON P Pediatrics in India in 1955. *Pediatrics 19* 317 1957
6. — Undergraduate pediatric education in India. *Indian J Pediat, 25* 293, 1958.
7 — Maternal and child health services in S.-E. Asia, II, III, IV (a) Afghanistan, *J Trop Pediat 2* 18, 1956, (b) Burma, *J Trop Pediat, 2* 84, 1956, (d) Indonesia, *J Trop Pediat, 2* 190, 1957
8. METTHOP G and ROBINSON P Maternal and child health services in S.E. Asia, V *J Trop Pediat 3*. 41 1957
9 KARUNARATNE, W A., ROBINSON P and TULL, R. L: Maternal and child health services in S.E. Asia, VI. *J Trop Pediat, 3*. 8., 1957

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Identification of Enteropathogenic *Escherichia Coli* 0111 B4 by Means of Fluorescent Antibodies

by DAN DANIELSSON and GUNNAR LAURELL

The bacteriological diagnosis of enteropathogenic *Escherichia coli* by conventional culture and the current serological tests takes 48–72 hours. In other words, the method is time-consuming, and furthermore requires special media. Moody Goldman & Thomason (11) showed in 1956 that, by means of Coons's (3) fluorescent antibody technique, it is possible to demonstrate pathogenic bacteria within about one hour. This technique has since been used for the identification of a number of different bacteria (1 4, 5 12, 13). Whitaker Page, Stulberg & Zuelzer (18) who examined 4-year old samples of faeces from an epidemic caused by 0127 B8 demonstrated the superiority of the method for diagnosing enteropathogenic *E coli*

In the investigation now described the fluorescent antibody technique was tested during a current epidemic of infantile diarrhoea due to *E col* 0111 B4 and was compared with the conventional bacteriological and serological techniques.

Methods

1 *Preparation of Antiserum and Conjugation with Fluorescent Substance*

Rabbit antiserum specific for enteropathogenic *E coli* 0111 B4 was prepared in accordance with the directions of Edwards & Ewing (7). The O-agglutinin titre varied from 1 1280 to 1:2560 and the B-agglutinin titre was about 1 124 These sera were used for the serological tests and for conjugation with fluorescent substance Before conjugation the globulin fraction was precipitated with 33%-saturated ammonium sulphate. The precipitat was dissolved in distilled water dialysed for 12 hours against running distilled water and then for a further 24 hours in phosphate-buffered sodium-chloride solution. Conjugation was performed by the method described by Chadwick, McEntegart & Nairn (2) using Lissamine Rhodamine B200 (RB200) which gives a brilliant orange fluorescence. It proved possible to dilute the conjugated globulin solutions 4–5 times with physiological saline without impairing the results. The conjugat was stored in small bottles fitted with screw caps at 4°C, or frozen at −20°C.

Treatment of the Smears

Each smear was dried in air and fixed by heating A small drop of conjugated globulin solution was spread out so as to cover the smear and the slide was placed in a damp chamber at room temperature. After a period of 30–45 minutes the slide was washed in phosphate-buffered saline (pH 7 1) for 10–15 minutes. The smear was mounted under a cover-slide in buffered glycerine-saline (pH 7 1). Control test of positive smears were made on conjugated normal

rabbit globulin, and also by means of inhibition tests (Goldman's one-step procedure (8)).

3 Fluorescence Microscopy

A Zeiss fluorescence microscope equipped with dark-field condensor and mercury lamp HBO200 was used. The BG1 ultraviolet input filter in combination with the OG4 or OG5 eyepiece filter (Schott & Gen) gave the best results. The following scale was used for reading off the count of fluorescent bacteria:

Negative	No fluorescent bacteria present
1 +	Isolated fluorescent bacteria present
+	About 1 fluorescent bacterium present per field
3 +	2–5 fluorescent bacteria present per field
4 +	5–10 fluorescent bacteria present per field
5 +	Numerous fluorescent bacteria present per field

4 Treatment of the Specimens of Faeces

The specimens were as a rule examined 24 hours after collection. Each specimen was treated as described below and was then stored at −15°C for subsequent examination (see Fig. 1)

Modification 1 Direct culture on Conradi Drigalski's "blue agar plates (9) After incubation at 37°C for 20–24 hours at least 12–15 colonies were examined by means of the slide agglutination test. Suspect colonies were pure-cultured in broth, and confirmed by means of O titration in tubes in accordance with current technique

Mod ficat on As described by Whitaker *et al* (14), a small quantity of faeces was suspended in 1 ml of physiological saline in sterile centrifuge tubes Of this, smears were made on slides measuring about 2 cm for treatment with fluorescent antibodies and fluorescence microscopy and direct cultures were also made as described under Modification 1

Modification 3. A suspension in physiological saline (see Modification 2) was made up to 5 ml, and filtered through gauze to remove coarse particles. The filtrat was centrifuged at 3000 r p.m. for 10 minutes, and the sediment suspended in about 0.5 ml of physiological saline. Preparations and culture of this were then made in accordance with the directions under Modification 2 (Thomason *et al.* (13)).

Modification 4 A small quantity of faeces was cultured in 2.5 ml of broth at 37°C for 6–8 hours. A direct culture of the broth was made (see Modification 1) The broth was then centrifuged, the sediment suspended in 1 ml of sterile physiological saline and smears were made for treatment with fluorescent antibodies. Culture of this suspension commonly resulted in such prolific growth that it was difficult t find sufficiently isolated colonies.

The specimens of faeces were again examined after having been stored for 3 months at −15°C. In these experiments the specimens were first cultured in broth at 37° and 45°C for at least 24 hours (see Dixon (6)). The broth culture was then treated as described under Modification 4.

Material

1 The Epidemic in Question

During the autumn of 1959 there was an epidemic of infantile diarrhoea at Children Home near Örebro. At the time 26 babies aged from to 12 months were resident at the Home By the time the investigation was commenced at the end of October 1959 13 f the infants had had diarrhoea for several weeks. *E col* strain 0111:B4 was demonstrated among these and in two pparently healthy children, by means of the fluorescent antibody technique. Clinically the epidemic was relatively mild. Three of the children had severe diarrhoea, and were admitted to hospital, where neomycin was given successfully

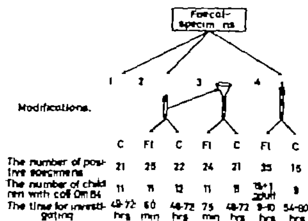


Fig. 1. Occurrence of *E. coli* 0111 B4 according to fluorescent antibody technique and conventional culture with the modifications used in this investigation.

C = Conventional culture. Fl = Fluorescent antibody technique

2 *Collection of Specimens*

During the first 2 months samples were taken every 4–5 days, and during the last 2 months every 10–12 days. Specimens were obtained from four of the healthy infants on only four occasions, at regular intervals throughout the period of the investigation. The staff, who numbered 24 supplied specimens on two occasions. Altogether 264 specimens of faeces were examined.

Results

The findings obtained by the various methods of investigation are shown in Fig. 1

The greatest number of positive results was obtained by the fluorescent antibody technique as described under Modification 4 Enteropathogenic *E col* of strain 0111 B4 were demonstrated in 15 infants on 23 occasions and in one of the nurses on one occasion. In comparison, the conventional method in the same modification yielded positive results in nine infants on 15 occasions. With regard to other methods, the fluorescent technique does not differ to any notable extent from conventional procedures Pathogenic bacteria were demonstrated on slightly more occasions by the fluorescent technique than by conventional methods, however

The quickest diagnosis was obtained by

TABLE 1 *Quantitative distribution of the positive results with fluorescent antibody technique and the number of positive cultures within each group.*

C = Conventional culture. Fl = Fluorescent antibody technique.

Modifications (see Fig. 1)		2		3		4	
		Fl	C	Fl	C	Fl	C
Quantitative gradation of bacteria per field	1+	11	8	13	10	7	0
	2+	10	9	10	9	2	1
	3+	2	2	1	1	4	2
	4+	1	1	1	1	5	0
	5+	—	—	—	—	17	12
No. of positive specimens		23	22	24	21	35	15
No. of children with *E. coli* 0111 B4		11	12	11	11	15 1 dult	9

TABLE 2 *Quantitative comparison between fluorescent antibody technique Modification 4 (enrichment) and all the positive cultures together*

C = Conventional culture FI = Fluorescent antibody technique.

Modifications (see Fig. 1)		FI 4	C 1,2,3,4
Quantitative gradation of bacteria per field	1+	7	0
	2+	2	1
	3+	4	4
	4+	5	4
	5+	17	17
No. of positive specimens		35	26
No. of children with *E coli* 0111 B4		15 + 1 adult	12

the fluorescent antibody technique without enrichment. It was in this manner possible to establish the presence of *E coli* 0111 B4 within one hour With enrichment, which gave the greatest number of positive cultures, this took 9–10 hours By the conventional procedure it was impossible to obtain a definite diagnosis in less than 48 hours.

Table 1 shows the bacterial counts as obtained by the various modifications of the fluorescent antibody technique compared with corresponding cultures It will be apparent that the fluorescent technique is the more sensitive Several specimens that on culture gave negative results yielded 1 + to * + on examination by the fluorescent antibody technique Only *one* specimen, which was found by Modification 2 to contain strain 0111 B4 gave negative results with the fluorescent antibody technique The difference was more striking after culture in broth. In none of the seven cases in which the fluorescent technique gave a result of 1 + were organisms of strain 0111 B4 isolated by culture Of 17 cases which yielded 5 + 1* gave positive cultures.

Table 2 shows the total number of *positive cultures obtained by the various* methods, and these are compared with the fluorescent antibody technique Modification 4 which gave the greatest number of positive results. Altogether positive results were obtained in nine more cases by the fluorescent technique than by conventional methods. In four of these nine bacteria of strain 0111 B4 had been obtained by culture on earlier occasions. Enteropathogenic *E coli* were demonstrated in a further three infants and one adult by the fluorescent antibody technique, compared with the cultures. The number of positive specimens from these cases was thus five.

Antibiotic Therapy

The importance of antibiotic therapy is apparent from Table 3 All infants at the Home were treated for 5 days. Specimens of faeces were taken from them all on the fourth day of treatment By standard culture *E coli* of strain 0111 B4 were isolated from two infants on that occasion. By Modifications 2 and 3, culture procedure positive results were obtained from three infants, whereas pathogenic bacteria were demonstrated in four by the fluorescent antibody technique The greatest difference between the methods is seen in Modification 4 conventional culture gave positive results in three cases, whereas the fluorescent procedure revealed pathogenic organisms in nine After antibiotic therapy no positive cultures were obtained in Modifications 1 and 4 but the fluorescent antibody technique in Modi-

fication 4 revealed the organisms in two infants. In one child *E. coli* of strain 0111 B4 were demonstrated by both techniques in Modifications 2 and 3

Semiquantitative Analysis

Laboratory tests were carried out to examine the sensitivity of the fluorescent antibody technique. For this purpose specimens of faeces of a diarrhoea consistency that had yielded positive results in Modifications 1–4 by both techniques were selected. These specimens were diluted with suitable negative faeces, and each dilution was examined by Modifications 1 * and 4. The results are collected in Table 4 It can be seen that after culture in broth bacteria were demonstrable by the fluorescent antibody technique in a dilution of 1/128 whereas the greatest dilution after which *E. coli* 0111 B4 could be detected by other techniques was 1/2.

Survival Power of the Bacteria

All specimens of faeces were re-examined after 3 months storage at −15°C Culture in broth at 37° and 45°C was performed before plating out. The results of the final cultures are shown in Fig. 2, from which it is clear that the fluorescent antibody technique yielded a greater number of positive cultures. An unexpected finding is that better results were obtained after incubation of the preliminary cultures at 37° than at 45°C

Discussion

The fluorescent antibody technique was tested during a current epidemic of

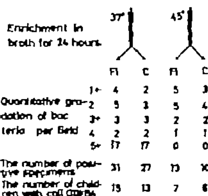


Fig 2. Secondary investigation of frozen faecal specimens after primary enrichment in broth t 37°C and 45 C for 24 hours.

C – Conventional culture. Fl – Fluorescent antibody technique.

infantile diarrhoea caused by enteropathogenic *E. coli* of strain 0111 B4. After culture in broth it was possible by means of this technique to demonstrate the organisms in 15 infants and one adult, whereas conventional culture revealed them in only 12 infants.

The comparatively small difference between the results of the two methods may possibly be ascribed to the fact that all specimens were taken during an epidemic Under such circumstances the incidence of bacteria in the faeces is as a rule high, and the organism can readily be demonstrated on culture. When the bacterial count is low the fluorescent antibody technique is probably superior Evidence in favour of this is supplied by the four positive fluorescence tests in which the strain could not be demonstrated simultaneously although the organisms had been present in earlier tests; and further support is gained from the results obtained during and after antibiotic therapy The greater sensitivity of the

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Serial Blood Gas Tensions and Acid Base Balance during the First Hour of Life in Human Infants[1]

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Although there have been a number of biochemical studies performed on umbilical cord blood samples, very few serial studies have been made during the first hour of life—the period in which many of the critical respiratory and circulatory adaptations to extrauterine life occur (22).

In the present investigation acid base balance of arterial blood has been followed serially with studies beginning within five minutes of delivery in the majority of subjects and continuing through the first hour. In addition the partial pressures of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) have been directly determined. It was hoped that PaO_2 in particular would add to the knowledge of this important period since in general it more precisely defines hypoxemia than does oxyhemoglobin saturation and thus might give information regarding the effectiveness of the lungs to oxygenate the blood as well as the presence of extra pulmonary right to left shunts.

Material and methods

Forty infants on the clinical obstetrical service of the Ohio State University Health Center were studied. All except three were mature by birth weight (these 3 weighed 2495, 2407 and 2280 g). In 27 the pregnancy labor and (vertex) delivery were considered entirely normal: 11 mothers had forceps applied at the outlet to assist delivery while in the remainder (16) delivery was spontaneous. Nine infants were delivered by elective cesarean section and four mothers had abnormalities either in maternal health (2) or in presentation (2). With few exceptions the mothers received a combination of meperidine and phenergan i.m. or i.v. during labor. The usual dose was 50 mg of each and, with few exceptions, they received this only once. No differences in results were observed in the groups who did or did not receive analgetic drugs. Anesthesia for the vertex deliveries consisted of inhalation (N_2O or GOE) in 15, conduction (spinal or caudal) in 10, local or none 1 each. All the patients delivered by cesarean section received conduction anesthesia. All infants were rated by the Apgar scoring method one minute after complete delivery (1).

Immediately after delivery the umbilical cord was doubly clamped and heated. The cord was then successively swabbed with an organic iodide preparation (Virak) and alcohol and then was amputated within 2 cm of the abdominal wall, in the meanwhile having an assistant compress the cord at the abdominal wall to prevent bleeding. After shallow dilatation of one of the arteries a

[1] Supported in part by a grant from the Comley-Coleman Fund of the Ohio State University.
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catheter[1] was inserted a distance of 4–10 cm. When it was technically not possible t cannulate an artery an attempt was made to catheterize the left atrium through the um bilical vein using a soft, round tip polyethy lene catheter marked at 1 cm intervals from 10–15 cm. Since this was a blind procedure, although recognized as technically easy because of the proximity of the foramen ovale t the inferior vena cava (10), it was possible to be certain that left atrial blood was obtained only when the catheter was 12–15 cm from the abdominal wall and the sample had a distinctly brighter color than blood obtained after withdrawing the catheter 1–2 cm. The results of left atrial samples are therefore somewhat weighted. Rectal temperature was recorded immediately after each sample was obtained.

The protocol called for a sample in the first five minutes of lif (successful in 24 infants) and serially thereafter at 10 20, and 60 minutes. In fact, there was considerable deviation from the protocol and the results were grouped int the time periods 2–5, 6–10, 11 20 21–40, and 41–64 minutes. Twenty-eight infants had at least 3 samples withdrawn, 9 had 2 and 3 infants only 1.

Each sample 1.5–2.0 ml in volume, was collected in lightly oiled syringe, the dead space of which was filled with heparin. A small amount of mercury was added after collection to assure mixing, following which the syringe was placed in crushed ice until analysis. Previous studies in this laboratory have shown that gas tensions, pH and CO_2 content are unaffected by adding heparin or mercury The blood gas tensions, pH, CO content and hematocrit determinations were begun within ½–2 hours after collection. The sera were frozen under mineral oil for subsequent determination of the concentration of chloride, sodium, potassium, magnesium and total protein. All chemical determinations were performed in duplicate. Blood gas tensions were determined by a micromodification of the blood bubble equilibration technique (4). Carbon dioxide content and pH were determined on plasma, obtained by centrifuging blood under a thick layer of neutral mineral oil. A Kopp-Natelson microgasometer was used for determining CO content (25). Plasma pH was determined at 37.5 C. using a modification of the Beckman microglass capillary and a Beckman model GS pH meter (47). Sodium and potassium were analyzed on a flame photometer with an internal lithium standard, chloride was determined by a titritometric method (34); magnesium by a micromodification of the method of Basinki (3); total protein by the biuret method (14) Electrolyte determinations were not performed on hemolyzed specimens. Sera of known chemical composition[4] were analyzed with each batch of patient sera.

Calculations

The partial pressures of O and CO were calculated to dry ambient pressure, T 37.5 C then corrected to body temperature (7). Oxygen saturation was obtained from the fetal oxyhemoglobin dissociation curve (31) using the corrected PO_2. The pH values were corrected to body temperature using Rosenthal' factor for whole blood (0.0147 units/°C). Calculated PCO_2, t compare with direct PCO_2, was derived from the Henderson–Hasselbalch equation; pK' was determined from the nomogram of Severinghaus *et al* (35). Buffer base (38) or "base excess" (2), which are useful methods of assessing metabolic disturbances, were not calculated in these infants for two reasons. Hematocrits, in general, were performed only on the initial and final blood samples and then not consistently Secondly the Singer Hastings nomogram for calculating buffer base assumes serum protein concentration of 7.2 g/100 ml, a pK 6.11 for carbonic acid and a

A No. 5 arterial catheter was found to be most satisfactory Polyethylene catheters tend to kink during insertion because of arterial spasm.

The senior author is indebted t Dr L. S. James who discussed and demonstrated the umbilical catheterization techniques with him.

Details of all methods (except Mg) are available in the Manual of Clinical Chemistry the Children Hospital, Columbus, Ohio, S. Meites, Ph.D., editor

Lab-trol, Dade Reagents Inc., Miami, Fla.

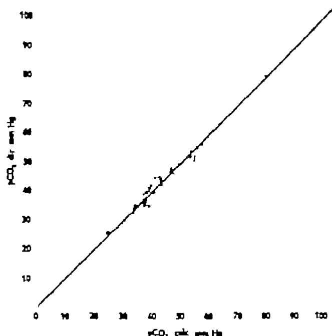


Fig. 1 Comparison of direct with calculated $PaCO_2$. Solid line is the line of identity. Dots ( ) infants in present study. Crosses ( ) infants, children and adults (4). A close correlation below $PaCO_2$ 65 mm Hg is present.

CO solubility factor of 0.0311. All three assumptions tend to be incorrect during the neonatal period: serum protein concentration is reduced; marked variations of pK′ and solubility occur as a result of temperature and pH shifts, which of course, may be profound during the first minutes and hours of life. It is believed that the tabular plus the graphic (9) representation of the relationship of pH, PCO and CO content are as helpful in these circumstances as buffer base. Attention is also drawn to the fact that slight loss of CO from the plasma through the overlying mineral oil may occur at high CO tensions; this will result in a decrease of calculated PCO compared to direct PCO_2. This does not occur when the PCO is less than 65 mm Hg (Fig. 1).

Results (Table 1)

Blood Gas Tensions

As expected, the partial pressure of oxygen was very low and carbon dioxide markedly elevated at birth. During the succeeding minutes the values tended to approach normal limits, although wide variations occurred in both PaO_2 and $PaCO_2$ during the entire hour (Figs. & 3). No statistical difference was found in the PaO_2 of infants born by vertex versus those delivered by cesarean section except at the 11–20 minute period (Table 3).

The PaO_2 in left atrial blood was uniformly higher than from the artery. In part this represents the biased selection of LA samples but it also clearly indicates the presence of a right to left shunt through the ductus arteriosus. This was documented in one instance when samples were simultaneously obtained from LA and artery with values of 72.0 and 57.4 mm Hg respectively.

Only four oxygen tensions were above

Table 1 *Serial biochemical data during the first hour of life*

Determination		Time period (minutes)				
		2–5	6–10	11–20	21–40	41–64
PaO_2 mm Hg	No.	23	22	28	1**	29
	Mean	19.5	43.7	54.3	56.7	61 7
	SD	12.3	15.9	12.1	7 7	13.8
	Range	6.6–47.3	29 7–65.1	37.5–79.8	46.. 72.5	40.1–89.5
$PaCO_2$ mm Hg	N	24	23	31	24	30
	Mean	6.4	57.2	46.3	39.2	34.0
	SD	11.6	13.0	8.3	.4	4.9
	Range	55.4–96.6	40 0–94.9	28.5–79.0	21 7–55.0	18.4–56.3
pH	N	23	21	31	4	30
	Mean	7 10	7 19	7.25	7.31	7.34
	SD	.09	.05	10	06	.06
	Range	6.94–7.22	7 00–7.30	6.94–7 43	7 14–7.5..	7.23–7.45
CCO_2 mM/L	No.	24	1	31	24	30
	Mean	24.5	23.3	20.8	20.6	21.6
	SD	2.8	..9	..8	2.4	..3
	Range	17 4–29.3	18.6–26.6	14..–24.8	16.1–23.7	16.6–*6.
Na mEq/L	No.	19	18	21	18	4
	M	139	136	133	135	134
	SD	8	7	5	4	5
	R	130–155	1..3–148	120–141	130–145	120–140
K mEq/L	No.	19	18	21	18	24
	M	9 1	8.4	8.0	8.5	8.0
	SD	2.0	1.8	1.8	1.7	1.8
	R	5.5–14 3	5.9–12.0	5.6–12.7	6.4–11.4	5.3–11.4
Cl mEq/L	No.	11	10	13	12	20
	M	107	107	100	108	109
	SD	4	4	5	3	4
	R	99–110	102 111	98–115	10*–113	101 113
Mg mEq/L	No.	8	2	6	9	14
	M	2.0	2.1	2.5	1.8	2.0
	SD	—	—	—	0.5	0.3
	R	1 4–2 7	2.0–2..	2 0–2.9	1.6–2.9	0.9–2.3
Total Protein gm/100 ml	No.	18	15	18	16	22
	M	6 4	6.5	6.4	5 9	6.1
	SD	0.8	0 6	0.8	0.6	0.9
	R	5 2–7 8	5.5–7.3	5.7–8 7	5.0–7 7	4.8– .0
Hematocrit	No.	14				15
	M	51	—	—	—	50
	SD	5	—	—	—	4
	R	38–58	—	—	—	41–57

* LA samples not included.

the lower limit of normal for this laboratory (88 mm Hg) Two of these were LA samples and two were from the artery Ten values were greater than 3 mm Hg and these were restricted to six infants thus demonstrating the variability with which this phase of extrauterine adaptation occurs.

During the period ..1–40 minutes nine of the twenty-one samples were from the left

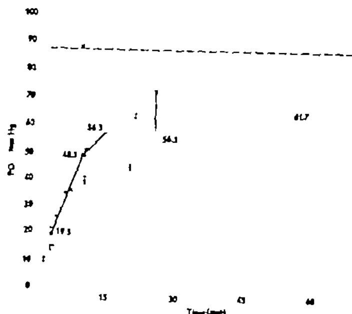


Fig. .. PaO versus time The figures are the means of the five time periods. The dots indicate arterial samples. The crosses indicate left atrial samples. The dashed line is the lower limit of normal PaO for older children and adults (88 mm Hg).

TABLE 2 *Acid-base balance in infants 1–3 days of age (Mean value.)*

Author	pH	CO_2 mM/L	pCO_2 mm Hg
Graham & Wilson	7 43	18.7	30.0
Cook *et al.*	7 44	1 9	31.2
Stahlman	7.43	21.9	32.7
Weisbrot *et al*	7 41	21 4	33.6
Reardon *et al.*	7.39	21 1	34 1

atrium (Fig 2). For this reason these values were not included in the calculation of the mean PaO_2 for this time period (Table 1) During the other periods the atrial values were included since they were few in number and did not significantly alter the mean. The mean PaO_2 at 2–5 minutes was 10.5 mm Hg ± 12.3 Although the lowest observed value (0 6 mm Hg) occurred in an infant whose mother required heavy sedation, values of 0 8 and 0 9 mm Hg were observed following entirely normal vertex deliveries. The two highest values, 47.3 and 35 5 mm Hg, occurred following vertex and section deliveries respectively

During the 0–10 minute period the largest increment of change in mean values occurred. PaO_2 rose to a mean of 45 7 mm Hg an increase of 20.. mm Hg During the same period $PaCO_2$ fell 19.. mm Hg to a mean of 57.2 mm Hg During the next ten minutes PaO_2 rose to a mean of 50.3 mm Hg and $PaCO_2$ fell to a mean of

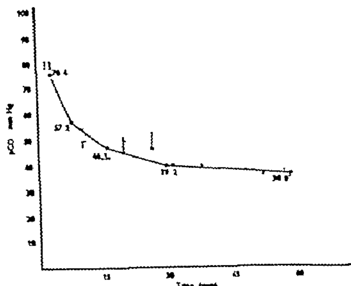


Fig. 3. $PaCO_2$ versus time. Symbols as in Figure 2.

TABLE 3 *Serial acid-base balance in infants delivered by vertex and by cesarean section*

	5–6 No.	5–6 Mean (SD)	6–10 No.	6–10 Mean (SD)	11–20 No.	11–20 Mean (SD)	21–40 No.	21–40 Mean (SD)	41–64 No.	41–64 Mean (SD)
pCO_2 mm Hg										
Vaginal	16	74.4(± 8.4)	17	64.0(± 9.8)	20	41.9(±9.2)	18	40.6(±7.2)	21	37.1(±6.0)
C.S.	6	81.2(±13.7)	3	55.0(±10.9)	6	[illegible]	6	37.0(±8.6)	7	41.2(±7.8)
P		0.1 > p > 0.05		> 0.1		< 0.01		> 0.1		> 0.1
pH										
Vaginal	18	7.12(±0.07)	16	7.31(0.07)	20	7.29(±0.07)	18	7.31(±0.06)	21	7.33(±0.03)
C.S.	6	7.04(±0.03)	3	13(0.06)	5	7.18(±0.13)	6	7.34(±0.12)	7	7.31(±0.06)
P		< 0.05		0.1 > p 0.05		< 0.01		> 0.1		> 0.1
CCO_2 mM/L										
Vaginal	16	25.1(±2.3)	17	22.2(±2.2)	20	20.9±(2.8)	18	21.1(±2.3)	21	21.8(±2.6)
C.S.	6	22.2(+2.1)	3	20.6(±4.6)	6	20.4(±3.4)	6	[illegible]0.6(±2.1)	7	21.7(±2.1)
P		0.05		> 0.1		> 0.1		> 0.1		> 0.1
pO_2 mm Hg										
Vaginal	15	22.0(±12.8)	17	52.2(±16.1)	18	59.0(±12.2)	15	64.6(±6.4)	20	62.8(±10.5)
C.S.	6	16.0(±10.2)	3	38.0(±13.6)	7	47.1(± 8.8)	6	55.7(±4.0)	7	57.7(±12.1)
P		> 0.1		> 0.05		< 0.05		> 0.05		> 0.1

46.3 mm Hg. During the remainder of the first hour there were only slight changes in these values: mean PaO_2 rose to 61.7 mm Hg and $PaCO_2$ fell to 38.0 mm Hg at one hour of age. The calculated mean percentage oxyhemoglobin saturations during these periods were 27, 83, 90, 9[illegible] and 95%.



Fig 4 The relationship of continuous administration of oxygen to mothers upon their infant's PaO within 5 minutes of birth. No effect is apparent. Symbols () normal vertex delivery; ( ) cesarean section; (O) abnormality in pregnancy or delivery

The effect of administration of oxygen to mothers

Blood was obtained within 5 minutes of delivery in fourteen infants whose healthy mothers received oxygen by face mask continuously for periods of 2 to 30 minutes prior to delivery Their PaO_2 was compared with nine infants whose mothers received no O_2 (Fig 4) No effect of maternal hyperoxygenation upon the infant s oxygen tension was found.

Acid-base balance

In considering acid base balance it is important to remember that both neonates (8 15, 30 41 45) and pregnant women (6 *6 44) have a reduction in $PaCO_2$, CO_2 content and buffer base Thus by normal adult standards these individuals would be considered to have a mixed metabolic acidosis and respiratory alkalosis. The present results must therefore be compared with normal neonates values not those of adults. Values of pH CO_2 content and PCO_2 for healthy infants one to three days of age obtained by several investigators are shown in Table 2.

Differences in acid base balance between infants delivered vaginally and by cesarean section were noted during the first 20 minutes (Table 3 Fig 5). The mean values of the twenty nine normal infants indicated an essentially pure respiratory acidosis which continued to improve during the hour although improvement was principally achieved in the first 20 minutes. Even at one hour however the $PaCO_2$ was still above normal. In contrast to these infants the infant born by cesarean section had a moderate metabolic acidosis in addition to a somewhat more severe respiratory acidosis. Furthermore the pattern of recovery was delayed and it was not until 21–40 minutes that the mean values approached the vertex delivered infants

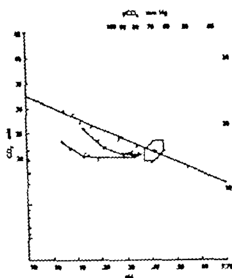


Fig. 5.

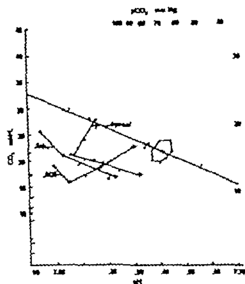


Fig. 6.

Fig. 5. Serial acid-base balance in normal vertex (open symbols) and cesarean section deliveries (solid symbols). The cross (+) is the normal mean for infants 1–3 days of age and the surrounding hexagon represents 1 standard deviation. The symbols indicate time periods (O), 2–5; (△), 6–10; (□), 11–20; (▽), 21–40; and ◎ 41–64 minutes.

Fig. 6. Serial acid-base balance in 3 infants with post partum respiratory distress. Abbreviations: RDS, respiratory distress syndrome; Asp, aspiration of amniotic fluid; Apnea? apnea of unknown origin. Symbols as in Figure 5.

Electrolytes protein and hematocrit

No significant differences in the concentration of serum electrolytes were noted between infants born by vertex or cesarean section. No changes with time were found in the concentrations of chloride sodium, or magnesium. Serum chloride values were higher than adults an observation previously noted by Graham *et al* (16). Serum potassium levels were strikingly elevated by adult standards the mean at –5 minutes being 9.1 mEq/L, falling at 41–64 minutes to 8.0 mEq/L. The range of values was wide and a few extremely high ones were noted. For example during the first five minutes 6 infants delivered by vertex had a concentration greater than 10.0 mEq/L, two of these had reduced Apgar scores (5 & 6) but the remaining four infants had scores of 8 or higher. The serum magnesium values were consistently within the range of adult normal (1.5–3.0 mEq/L).

The mean serum total protein concentration for the entire group of children ranged from 6.4 g/100 ml at birth to 6.10 g/100 ml at one hour. The infants born by vaginal delivery had a significantly higher protein concentration during all except the 21–40 minute period than infants born by cesarean section (Table 4).

The mean value for hematocrit during the initial and final periods were 51 and 50%, respectively. Unfortunately too few determinations were performed in the cesarean group for statistical comparison

TABLE 4 *Serum protein concentration in vertex and cesarean section deliveries (g/100 ml)*

Time period (min)	Vertex	C.S.	P
–5	6.7 (±0.42)	5.6 (±0.26)	<0.01
6–10	6.6 (±0.45)	5.3 (±0.16)	<0.01
11–20	6.7 (±0.73)	5.6 (±0.23)	<0.05
21–40	6.1 (±0.60)	5.5 (±0.54)	0.1 > p > 0.05
41–64	6.4 (±0.28)	5.1 (±0.38)	<0.01

TABLE 5 *Comparison of biochemistries in infants with Apgar scores of 5–7 and 8–10 (Mean values)*

	Score	Time period (minutes) –5	6–10	11–20	41–64
PaO mm Hg	5–7	16.1	45.0	51.5	63.9
	8–10	22.8	51.9	57.5	60.5
PaCO mm Hg	5–7	78.6	63.0	46.5	35.3
	8–10	74.7	51.7	40.4	39.9
pH	5–7	7.08	7.15	7.23	7.35
	8–10	7.11	7.22	7.25	7.33
K mEq/L	5–7	9.8	8.7	7.9	8.3
	8–10	8.9	7.9	8.0	7.9

since a difference from the normal infants was suggested. In the former group the values of the three hematocrits performed during the first 5 minutes were 38, 46, and 49 %. In 11 infants born by vertex presentation all except two (with values of 48 and 50 %) had values higher than 51 % at the 2–5 minute period.

Relationship of biochemistries to Apgar score

Thirty-nine of the 40 infants had scores of 5 or higher. Twenty-eight had scores of 8–10; these included 20 vertex deliveries, 5 infants of cesarean section and 3 with history of abnormalities during pregnancy or delivery. Eleven infants had scores of 5–7; seven were vertex and 4 were section deliveries. Differences in mean values of PaO_2, acid base balance and serum potassium concentration were noted in these two groups during the first 10 minutes of life (Table 5). By 11–20 minutes and thereafter no differences were apparent.

Discussion

Knowledge of the arterial oxygen tension is important primarily because the level indicates the head of pressure which forces oxygen into the tissues. The value cannot be derived accurately from the O_2 saturation at low or particularly high saturations (90 % or higher) because of the shape of the oxyhemoglobin dissocia-

tion curve. Furthermore unless body temperature and pH are taken into account large errors may be made by such calculations (36). This is very important in the period immediately following birth when both temperature and pH may be greatly altered.

The importance of knowing the oxygen tension is well illustrated by the values observed at 1 hour of age. Despite the fact that the mean oxyhemoglobin saturation was within normal limits (93%) the infants nonetheless had moderate hypoxemia as indicated by the mean PaO_2 (61.7 mm Hg).

Comparatively few investigations of oxygen tension have been performed in neonates and no serial studies have been reported. Sjöstedt *et al* (39) and Beer *et al* (5) determined the PaO from the clamped umbilical cord following normal vaginal deliveries and found mean values of 16.9 and 9.2 mm Hg, respectively. No studies are available to compare with the levels obtained during the first hour. Graham (17) has determined PaO_2 in infants 3 to 54 hours of age. He found a mean of 74 mm Hg (versus his normal for adults of 84 mm Hg). Reardon *et al* have recently reported (30) the results of arterial oxygen tensions on 24 infants ranging in age from 3 to 150 hours. They found a mean value of 82.7 mm Hg. The mean value for adults by the method these investigators employed is approximately 93 mm Hg. Thus it is apparent that there is a significant reduction in oxygen tension for several days of life even though the percentage saturation of oxyhemoglobin approaches or becomes normal promptly in some cases as early as 10 minutes after birth. There appear to be two reasons for the low tension, shunting through the ductus arteriosus and shunting within the lungs.

Although the principle direction of the shunt through the ductus arteriosus, which is physiologically patent for several days (42) is from left to right there is also some R–L shunt. This has been demonstrated angiocardiographically (16) as well as chemically. Eldridge & Hultgren (12) found a difference in oxygen saturation in arterialized capillary finger and heel blood during the first three days of life although these infants were deliberately stimulated to cry thus promoting a R–L shunt. Our studies show that left atrial samples have a consistently higher oxygen tension than arterial samples. This is true whether the infants are quiet or crying. The only conclusion permitted by these results is a right to left shunt through the ductus. However this does not explain the finding that there were only four values within the range of adult normal. The most likely explanation for this is a disturbance of pulmonary ventilation–perfusion relationships ("pulmonary shunting") presumably due to uneven ventilation. Graham has supporting evidence for this belief (17). Neonates and adults were given 50% oxygen to breathe the mean PaO of the adults rose to 270 mm Hg whereas the infant s rose to only 153 mm Hg. Furthermore since Reardon *et al* collected temporal artery samples a shunt through the ductus arteriosus cannot explain their reduced values.

The decreased PaO_2 is not due to disturbance in diffusion since this has been shown to be normal in newborns (41). A right to left shunt through the foramen ovale appears to be an unlikely explanation

for the reduced PaO_2. Although there is evidence (18, 23, 28) that a shunt occurs in some neonates during crying it has not been observed during quiet periods. We have made observations of PaO_2 during quiet followed by forced cry in 9 infants. In three there was a significant rise of PaO_2 (6, 14, 15 mm Hg) with crying; in three there was no change and in three a decrease of 10–18 mm Hg. These results suggest that changes occurring with cry are due to changes in ventilation as well as possible shunting through the foramen ovale or the ductus arteriosus.

Many obstetricians and anesthesiologists practice and teach the principle of having mothers breathe high oxygen concentrations during the second stage of labor with the view to increasing the oxygenation of the fetus and the newborn. Sometimes cited as evidence is the study of McClure (24) who observed a higher oxygen tension (mean 10 mm Hg) in umbilical venous blood in a group of infants whose mothers received oxygen compared to a control group. The objection to this study is that umbilical venous rather than arterial blood was analyzed. It is, of course, the latter that perfuses the tissues. Our study of oxygen tensions and James' of oxygen saturation (19) in arterial blood clearly demonstrate that continuous maternal oxygen administration of up to one hour's duration does not increase the infant's PaO_2 at birth. Whether or not the fetus is better oxygenated by O_2 therapy cannot be answered by these studies.

The failure of maternal hyperoxygenation to effect the infant particularly when it is considered with the acid base disturbance at birth appears to be best explained by the concept that the delivery process results in asphyxiation (20, 21). In the normal infants delivered by vertex presentation the acid base changes are essentially those of a pure respiratory acidosis. In depressed infants and, in our material, infants born by cesarean section, a metabolic acidosis is superimposed. In the normal babies the recovery from the acidosis was very prompt—occurring primarily in the first 10 minutes of life. In the section babies it took 21–40 minutes. James has pointed out that the recovery process is slower in more severely depressed infants. It may not only be slower but may assume a different pattern as well. Three infants who had serial blood collections developed respiratory disturbances. One of these developed, beginning at 20 minutes of age, typical clinical and later roentgenological evidence of the respiratory distress (hyaline membrane) syndrome. At birth his acid base balance was characterized by the most profound metabolic acidosis we observed in any of the infants in the study (Fig. 6). His initial ventilatory response as evidenced by a rise in PaO_2 and fall in $PaCO_2$ was quite normal and it was only at 1 hour that chemical evidence of disturbed ventilation was noted, by which time he had nearly recovered from the metabolic acidosis. This infant had mild clinical manifestations and was completely normal at 4 hours of age. In contrast to this infant, 2 others with respiratory depression at birth (one due to aspiration of amniotic fluid, the other of unknown cause) had a pure respiratory acidosis at birth, metabolic acidosis occurred some minutes later (Fig. 6), presumably as a result of a somewhat prolonged period of respiratory depression even though both infants were resuscitated

beginning within 2 minutes of delivery Although the data are suggestive, more studies beginning at birth in infants who subsequently develop evidence of the respiratory distress syndrome are necessary before one can conclude that the primary disturbance is metabolic rather than respiratory

The finding of hyperkalemia during the first hour of life adds to rather than settles the confusion on this point James (21) and Reardon (?9) have found only a slight elevation of potassium at birth in normal infants which is said to return to within normal limits within one hour although details have not been reported. Earl *et al* (11) reported a mean K concentration of 5.0 mEq/L in fresh placental venous blood. In contrast, Widdowson & McCance (45) observed a mean value of 8.0 mEq/L (range 4.8–12.0) in umbilical venous blood obtained immediately after delivery These discrepancies suggest technical errors and, indeed James has observed that the potassium concentration of umbilical venous blood obtained from the placenta rises sharply if there is a delay before the sample is obtained. However this objection does not apply to these studies It is well established that a fall in pH results in a shift of potassium from the cells to the extracellular space and a consequent rise in serum concentration (13 22, 27). This is true whether the reduction in pH is metabolic or respiratory in origin. When pH is controlled profound changes in PCO_2 or CO content do not cause a potassium shift (27) It is therefore not remarkable that serum potassium concentration should be elevated in newborns. However it has been shown in animals that it takes 1– hours for peak serum levels to be reached after the initiation of acute acidosis and this observation does not fit with the hypothesis that the period of asphyxiation is of short duration. Perhaps it is longer than we presently believe

Within the first hour potassium concentration begins to fall as the pH rises. This effect has been shown to occur in dogs in whom the ureters have been ligated and is due to a shift of potassium back into the cells (32) Pincus *et al* have observed modest elevation in potassium concentration (means of 6.1–5 4 mEq/L) in premature infants of varying weight groups in the first 24 hours of life (?7) Furthermore, in infants 1–9 days of age the mean level was 4.9 mEq/L and in only 2 to 15 infants was the concentration below the adult mean of 4 4 mEq/L (11) It thus appears that it may take several days for potassium to reach adult levels from the high levels noted at birth and in the first hour of life. Insofar as none of the infants manifested clinical evidence of hyperkalemia it is possible that they were protected by their coexisting hypercarbia (33).

Usher (43) has emphasized that hyperkalemia is a feature of the neonatal respiratory distress syndrome and has suggested a plan of management (44). His finding fits in well with the observations of a metabolic acidosis with further lowering of pH in this syndrome.

Several differences between infants born vaginally and by cesarean section have been observed in this study The decreased protein concentration in infants born by section has also been found by Reardon (20). The mechanism of this finding is unknown although she has suggested it

is due to hemodilution. A lower hematocrit in these infants is not entirely unexpected since factors which appear to be operative in the "placental transfusion" including uterine contractions (39) and elastic recoil of the chest (22) are not operative in section infants. In addition, if hemodilution does in fact occur in these infants it would contribute to the lower values. Further study is clearly indicated

Summary

1 Serial determinations of oxygen and carbon dioxide tension, pH CO_2 content sodium potassium, chloride magnesium, total protein and hematocrit beginning within 5 minutes of birth and continuing through the first hour have been performed in newborn infants.

2. The data support the concept that the delivery process is invariably asphyxiating whether delivery is vaginal or by cesarian section. Furthermore the major recovery occurs within 10 minutes of delivery in healthy infants.

3 Arterial oxygen tension was reduced throughout the entire period Even though the mean arterial oxyhemoglobin saturation was within normal limits at one hour of age the mean oxygen tension remained low The interpretation of these findings is discussed.

4 The administration of high oxygen concentration to the mothers for period up to 30 minutes failed to effect the infants PaO_2.

5 Evidence is presented for a right to left shunt through the ductus arteriosus when the newborn is quiet as well as crying

6 Serum potassium concentration was found to be considerably elevated in all infants. The mechanisms for this are discussed.

7 Differences in acid base balance serum protein concentration and packed cell volume between *infants delivered* vaginally and by cesarian section were noted. The explanation of these differences is not known.

Acknowledgement

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References

1 Apgar, V A proposal for new method of evaluation of the newborn infant. *Curr Res Anesth Analg* 3. 260, 1953.

2. Astrup P Jorgensen K Andersen O S. and Engel, K. The Acid base metabolism. A new pproach. *The Lancet* (in press).

3. B rvki, D H Reported t meeting of Amer Am Clin Chemists, Cleveland, Ohio, Aug. 29 1959

4 B tes G D. and Oliver, T K., J A micromodification of the bubble method for the direct determination of blood-gas tensions. *J Lab Cl Med* (submitted).

5. Beer, R., Bartels, H. and Raczkowski, H A.: Sauerstoffdissoziationskurve des fetalen Blutes und der Gasaustausch in der menschlichen Placenta. *Arch Ges Physiol*, 260 306, 1955.

6. Bouterline Young, H and Bouterline Young, E.: Alveolar carbon dioxide levels in pregnant, parturient and lactating subjects. *J Obstet Gynaec Brit Emp* 63 509 1956.

7 Bradley A.F Stupfel, M. and Severinghaus, J W Effect of temperature on pCO and pO of blood in vitro. *J Appl Physiol* 9 201 1956.

8. Cook, C. D Cherry R. B., O'Brien, D Karlberg, P and Smith, C. A.: Studies of respiratory physiology in the newborn infant. I. Observations on normal premature and full-term infants. *J Clin Invest* *34* 975, 1955.
9. Davenport H. W ABC of Acid-Base Chemistry The University of Chicago Press, 3rd ed., 1950.
10. Dawes, G. S.: Changes in the circulation at birth and the effects of asphyxia, in Recent Advances in Pediatrics, Interscience Publishers, Ltd, London, 1958, p 1
11. Earl, D P Barwin, H. and Hirsch, D The plasma potassium level in the newborn, *Proc Soc Exp Biol Med* *76* 756 1951.
12. Eldridge, F L. and Hultgren H. N Physiologic closure of the ductus arteriosus in the newborn infant. *J Clin Invest* *34* 987 1955.
13. Gimbeck, G Bergman, L. and Pitts, R. A.: The external response to acute acid-base disturbance of respiratory origin. *J Clin Invest*, *39* 231 1955.
14. Gornall, A. G Bardawill, C. J and David M. M.: Determinations of serum proteins by means of the biuret reaction. *J Biol Chem*, *177* 751 1949
15. Graham, B. D. and Wilson J C.: Chemical control of respiration in newborn infants. *Amer J Dis Child*, *87* 287 1954.
16. Graham, B. D Wilson J C., Tsao, M. U Baumann M. L. and Brown S.: Development of neonatal electrolyte homeostasis. *Pediatrics*, *8* 68, 1951
17 — In Adaptation to Extrauterine Life, Report of the thirtyfirst Ross Conference on Pediatric Research, Columbus, Ross Laboratories, 1959, p. 57
18. James, L. S.: idem p. 28.
19 — idem p. 70.
20. James, L. S., Weisbrot I. M., Prince, C. E., Holaday D. A. and Apgar, V The acid-base status of human infants in relation to birth asphyxia and the onset of respiration. *J Pediat*, *52* 379, 1958.
21 — Acidosis of the newborn and its relation to birth asphyxia. *Acta Paediat* *49* 17 1960, Suppl. 122.
22. Karlberg, P The adaptive changes in the immediate postnatal period with particular reference to respiration. *J Pediat* *56* 585, 1960.
23 Lind, J Changes in the circulation and lungs at birth. *Acta Paediat* *49* 39, 1960, Suppl. 122.
24. McClure, J H.: Newborn blood oxygen. A method of increasing the partial pressure of oxygen in the blood of the newborn infant. *Obstet Gynec*, *11* 686, 1958.
25. Natelson S.: Routine use of ultramicro methods in the clinical laboratory *Amer J Clin Path*, *21* 1153, 1951
26. Oard, H. C. and Peters, J P The concentration of acid and base in the serum in normal pregnancy *J Biol Chem*, *81* 9, 1929.
27 Pincus, J B Gittleman I. F Saito, M. and Sobel, A. E.: A study of plasma values of sodium, potassium, chloride carbon dioxide, tension, sugar urea and the protein base binding power pH and hematocrit in prematures on the first day of life *Pediatrics*, *18* 39, 1956.
28. Prec, K. J and Cassels, D. E.: Oximeter studies in newborn infants during crying *Pediatrics*, *9* 756, 1952.
29. Reardon H.: In Adaptation to Extrauterine Life. Report of the thirtyfirst Ross Conference on Pediatric Research, Columbus, Ross Laboratories, 1959 p. 79.
30. Reardon, H., Baumann M. L. and Haddad, E. J Chemical stimuli of respiration in the early neonatal period. *J Pediat*, *57* 151 1960.
31 Rooth, G Sjöstedt S. and Caligara, F The "in vivo" foetal oxygen dissociation curve. *Biol Neonat*, *1* 61 1959.
32. Schroeder, D. H., Prescott-Smith, K., Burrell, J M.: The effect of acute respiratory acidosis on the internal equilibrium of potassium. *J Clin Invest*, *39* 1276, 1955.
33. — Potassium intoxication during and immediately following respiratory acidosis. *J Clin Invest*, *33* 965, 1959
34. Schales, O and Schales, S. S.: Simple and accurate method for determination of chloride in biological fluids. *J Biol Chem*, *140* 879 1941
35. Severinghaus, J W Stupfel, M. and Bradley A. F Variations of serum carbonic acid pK with pH and temperature *J Appl Physiol* *9* 197 1956.
36. — Oxyhemoglobin dissociation curve correction for temperature and pH variation in human blood. *J Appl Physiol*, *12* 783, 1958.
37 Scribner, D H. and Avedon M.: Acid base alterations and plasma potassium concentration. *Amer J Physiol*, *197* 319 1959.
38. Singer, R. B. and Hastings, A. B.: An improved clinical method for the estimation of disturbances of the acid-base balance of human blood. *Medicine (Balt)*, *27* 223, 1948.
39 Sisson T R. C. and Whalen L. E.: The blood volume in infants. III. Alterations in the first hour after birth. *J Pediat*, *56* 43, 1960

No exact information is available regarding the normal cell excretion in the first days of life Nevertheless such information is imperative for the discussion of urinary findings in pathological conditions in neonates. It is the purpose of this paper to discuss observations on the cell excretion in the urine of normal newborn infants Specimens of preformed urine collected during or within minutes after delivery as well as specimens obtained during the first week of life were studied.

Material and Method

Most urine samples were obtained in the Obstetric Department of the University Clinic Rikshospitalet. A few were obtained in a general practice Only infants of healthy mothers with uncomplicated pregnancies and uneventful deliveries were included. Infants with respiratory distress of the slightest degree or with any known pathological trait were excluded. The neonates were examined by a pediatrician specially associated with the obstetric department as well as by the author The infants were fed according to modern principles of neonatal care (1) Only those with a normal weight curve and without signs of unphysiological dehydration were included in the study A total of 380 specimens obtained t one or two day intervals from 114 male and 27 female infants were studied. The preponderance of males reflects the convenience of sampling

A proportion of infant void immediately at birth, and alert midwives were able to collect some samples in the air These specimens were immediately stained for microscopic examination. Many infants void when unclothed, or when the suprapubic and perineal regions re being cleaned These specimens were easily collected for examination. Most samples, however were collected over 1 and 24 hou periods.

In the boys a plastic colostomy bag with a central hole surrounded by adhesive was fitted around the penis after thorough cleansing of the skin and prepuce To a oid artificial bleeding the prepuce was not withdrawn. In female infants a soft funnel made from one-half of a rubber ear syringe was fitted around the entire vulva and labia majoris after cleansing The funnel was held in place by adhesive band fixed to the abdomen and the gl teae exerting a firm pressure especially on the perineum. It was made certain that the anus was entirely free and outside this elastic funnel. The plastic bag was then fixed around the outer opening of the funnel When only examination of the sediment was to be performed a few drops of stain were placed in the bag before fixing in position, otherwise no st in was added at that time The stain described by Sternheimer & Malbin (18) was used. A few drops of formaldehyde were also added to secure good fixation of the cellular elements.

The plastic bag was i position for few hours, 1 hours or 4 hours according to purpose. The bag was emptied at intervals without being removed by cutting a hole which was sealed afterwards with adhesive bands.

Examination of all urines included pH specific gravity protein and sugar in addition to examination of the sediment. In some infant additional urinary studies (electrolytes and amino acid excretion, as well as capillary blood analyses of NPN creatinine hematocrit, electrolytes and CRP) were performed. These results will subsequently be published (5). Catheterization of female infants was not performed. It was felt that the use of the catheter should be restricted to more imperative occasions ( ). The risk of contamination of the urine by leucocytes from the vulva is relatively low in newborn female infant (4), although it is significant in some cases. Two girls with slight physiological vaginal bleeding on the 5th and 8th day after birth were excluded from the present material. Hematuria which can appear in normal boys was not observed in this study Because the

sampling from female infants was complicated by leaking at the perineal rim only males were used for timed urine collections.

The urine sample was thoroughly mixed, following which 5 or 10 ml were centrifuged for 5 minutes at 2000 r.p.m. The supernatant was decanted leaving a final volume of 0.1 the initial volume. One or two drops of stain were then added and shaken. The specimen was then allowed to stand for 15–20 minutes before examination. If kept longer the stained specimen was stored in a refrigerator at +4 C. The sediment was re-mixed then counted in a white-cell-counting chamber under both low and high power as well as by phase contrast in most instances.

The stain was used to ensure good fixation of the organic sediment, and also to distinguish the cell structures. It was especially valuable in the differentiation between leucocytes and renal epithelial cells (non-squamous epithelial cells). This differentiation is difficult and very often an impossible task even for experienced investigators (9). In this study differentiation was attempted by tabulating the cells in three categories: typical non-squamous epithelial cells of renal type, typical leucocytes, and cells of more uncertain structure. Often the last category was predominant. The total of the three categories was tabulated, epithelial cells other than those of assumed renal origin were not counted. All counts were performed two or three times and the mean tabulated.

Results

The results obtained from examination of the specimens from the 141 infants are illustrated in Fig. 1. One girl was omitted from the study because of a leucocyte count which exceeded 1500 cells per mm³. A catheterized specimen contained a number of cells similar to that found in most of the other infants and she was subsequently found to have a purulent vaginitis. The sediment counts from the other female infants did not differ appreciably from the males. Vaginal epithelial cells were present, but the leucocyte counts were not among the highest ones in this series of neonates. Accordingly sex differentiation was not pointed out in the figure. The average number of cells excreted *in the preformed urines was 10 per* mm³ and the highest number was 30 per mm³. One specimen was devoid of leucocytes and epithelial cells, although two erythrocytes were present. Most specimens contained 0–4 erythrocytes per mm³. Casts were found in six cases. In one specimen two casts were noted, while the other five contained only one cast per mm³. The casts were of the hyaline type with or *without epithelial* cells or *cell* debris incorporated. The specific gravity, pH, albumin and sugar content were similar to those found by others in preformed urines (14).

The great variance between infants in the combined counts of white and epithelial cells is clearly illustrated in Fig. 1. Both the average for each day and the normal range are tabulated in Table 1. *No specimen was free of cells,* in contrast to what has been observed by others (1, 4, 10). This is probably because the examination was performed on centrifuged urine in this study. Centrifuging here gives a *tenfold greater chance of discovering cells* in the 0–10 cell per mm³ range than does examination of uncentrifuged urine. For the first few days of life renal epithelial cells outnumbered the leucocytes in most infants. At the end of the first week the leucocytes tended to equal or outnumber the epithelial cells. The proportion between them could not be calculated because the uncertain group "leucocytes or epithelial

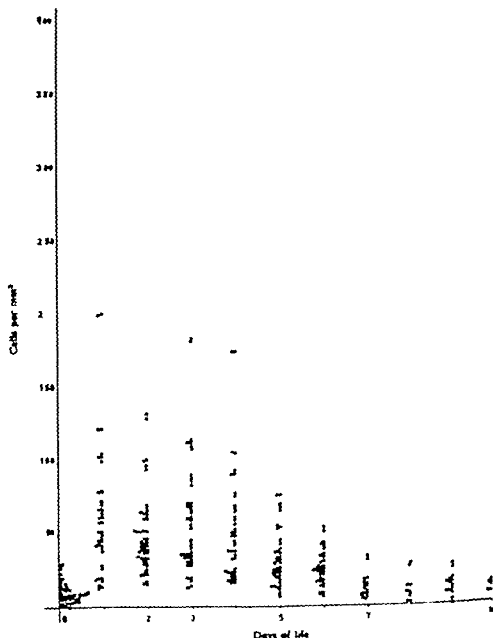


Fig. 1 Sum of epithelial cells and leucocytes excreted per cubic millimetre ... voided at birth, ... preformed urine ● other specimens.

cells was dominant in a majority of count An example of these variations is illustrated in Table 3 Great variability in count from specimen to specimen was observed in each individual and is magnified when the cell excretion i ex pressed as an Addis count.

The objections to the Addi count which have been made by several authors (6 17) also apply when the method is

TABLE 1 *Total leucocytes and non-squamous epithelial cells excreted per cubic millimetre of urine*

Day	Preformed urine	1	2	3	4	5	6	7
Average	10	78	65	67	58	43	37	28
Range	0–30	3–397	7–357	8–183	13–1 6	7 140	7–174	4–84
Number of specimens	33	45	50	50	42	45	37	21

adjusted for infants. Voiding is uncontrolled and appears at irregular intervals; furthermore, it is uncertain whether the bladder is completely emptied or not at each voiding. In addition the urine output is very variable. Some infants retain the urine in the bladder for long periods making considerable intravesicular cell lysis possible. A proportion of infants do not void at all in the first 48–72 hours of extrauterine life (19). Timed sampling is impossible without repeated catheterization. This difficulty was exemplified in one infant, who, during the 12 hour sampling period, voided only 4 ml. Just as the bag was removed and he was being washed he urinated 21 ml. The few minutes added to the sampling period would increase the Addis count six fold! In order to compare the sediment counts in the present study with those done by others, the Addis count has been calculated for infants from whom the collection procedure was optimal (Table 2).

The daily urine volumes computed from 12 hour periods, and partly by sampling for 24 hours are found in Table 4. When the results are compared with those of Thomson (19), a remarkable conformity is found. With the normal range in mind, it is understood that computing the 12-hour secretion of cells from theoretical volumes

TABLE 2 *The range and the average Addis sediment counts of cells and casts in the urine from newborn infants compared to those of normal children and adults*

R = range, A = average.

Series		Leucocytes and renal epithelial cells	Erythrocytes	Casts
Addis (adults)	R	32,000–1,835,000	0–4_5,000	0–4270
	A	322,000	65 30	1040
Lyttle (74 children)	R	9000–2,63_,000	0–120,000	0–12,916
	A	372,184	15,181	1083
Anoke (402 children)	R		0–800,000	0–20,000
	A		81,000	1230
Oiles (20 children)	R	9000–714,000	0–438,000	0–8330
	A	258,000	61,323	11 5
Author (119 specimens first 6 days)	R	42,000–12,500,000	0–830,000	0–440,000
	A	1,965,529	80,219	12,65_

TABLE 3 *Serial sediment counts from one male infant*

Cell type	Day 0		3	6	7
Leucocyte	1	30	4	9	4
Non-squamous (renal) epithelial	3	48	27	6	3
Uncertain	5	13	20	7	3
Total	9	91	51	22	12

TABLE 4 *Average and range of daily volume of urine Computed from 1 -hour and 1 hour specimens The corresponding figures of Thomson (19) in brackets*

Day	Volume ml	Range ml	Number of infant
1	16.8 (19.5)	0–88 (0–68)	32 (33)
	34.1 (20.6)	0–84 (0–82)	30 (20)
3	63.6 (36.0)	0–71 (0–96)	7 (26)
4	86.4 (64.8)	14–160 (5–180)	27 (26)
5	1_3.6 (103.3)	44–216 (1 17)	17 (23)
6	134.8 (124 6)	14[*] 260 (4_ 265)	16 (19)

1 -hour specimen, the next lowest was 40 ml in 4 hours.

as done by Snoke in his study (15) will give erroneous results in neonates. Accordingly it is not attempted in the present paper

Of the 380 specimens examined, 145 contained erythrocytes. Red blood cells were most abundant during the first days of life and subsequently declined in number After the first week they were sparse In dilute and alkaline urines erythrocytes disintegrate very quickly making the examination of the newly voided urine imperative unless fixation i used. This also applies to casts Casts were found in almost every specimen They were mostly of the granular type or hyaline with epithelial cells or cell debris incorporated Occasional epithelial ca ts were seen In a few casts one or two typical leucocytes were found incorporat l id by side with renal pithelial cells Three infants with such casts were followed for 8–14 weeks No sign of renal disease or urinary infection was found the infants thrived perfectly and all follow up urine specimens have been normal. The Addis counts for erythrocytes and casts in the newborn infants are compared with the similar figures published by others (4 10 1) in Table 2.

The method of counting the cell in a hemocytometer was compared with the more conservative method of judging the cell excretion by high power field microscopy (Table 5) In the present study the comparison applies to centrifuged urine Conformity is found with the result published by Stansfeld & Webb (17), who studied uncentrifuged urine

Some specimens were studied to determine the degree of cell lysis which occurred when the urine remained in the pla tic

TABLE 5. *Comparison of cell excretion expressed as cells per mm³ to high power field microscopy*

Cells per mm³	Cells per h.p.f.
400–	10–50 (numerous, heaps)
200–400	8–20
100–200	5–10
50–100	16
20–50	0–1 occasional
0–20	None occasional

TABLE 6. *Number of cells remaining intact after 3, 6, 12 and 24 hours. Unstained specimens at body temperature. Stained specimens at +4°C*

Specimen	S = stained U = unstained	Hours 0	3	6	12	24
A.N	S	22		21	23	20
	U	22	17	18	15	9
J.T	S	4	27	23		23
	U	24	23	19	14	10
E.E.	S	239	252		280	320
	U	279	311		254	173
M.P	S	62		60	85	66
	U	42		85	23	31
E.P	S	94	91	91	106	89
	U	94		70	84	57

bag for some time. Specimens were obtained immediately after voiding. Five ml were examined and the remaining urine was placed in a plastic bag close to the infant beneath the diaper. Other specimens were stored at 37 C. At intervals of 3, 6, 12 and 24 hours 5 ml aliquots were examined. The progress of cell lysis after staining and storing in a refrigerator at +4 C was studied in a similar way. The results of the lysis study are found in Table 6. The cells undergo a very significant lysis when kept in the plastic bag at body temperature. A similar process of cell lysis also probably occurs when the urine is retained in the bladder. Disintegration progresses were fastest in erythrocytes and hyaline casts. The epithelial cells were the most resistant. The lysis was most prominent in dilute urines and at a high pH. The degree of lysis in unstained specimens corresponded fairly well with that found by Houghton and Spears in urine from adults (6). The cells were not influenced much by lysis when the urine was stained as described and stored in a refrigerator. This is a practical point for the clinician who is not always able to

study the urine specimen under the microscope immediately after the specimen is produced. For studies of urine sediment only a few drops of stain may be placed in the bag before it is positioned.

Discussion

It has long been known that the urine of the normal newborn infant contains a higher proportion of cells and casts than that found in older children and adults (3, 11) This is confirmed by the present study It was also observed that the sediment count in normal neonates often exceeds the limits for children and adults established by earlier investigators. This is true for erythrocytes and casts and even more so for leucocytes and non squamous epithelial cells assumedly of renal origin. In the first days after birth the infant s urine is rich in non squamous epithelial cells which are believed to originate from the lining of distal tubules and collecting ducts The abundance of such cells in the urine is probably a result of a rapid growth of the epithelial lining with a lively replacement of cells The presence of numerous leucocytes suggests that blood cells enter the lumen through the epithelial lining possibly through small transient defects in thi lining under normal conditions. The demonstration of leucocytes in casts from some neonates proves that at least some leucocytes enter the urine proximal to the collecting tubes, a these cast were not of the broad type In older children and adult the presence of leucocytes in ca t i suggestive of a pathological condition (0 13) In neonates however the passage of leucocytes through the epithelial lining i probably made possible by the exfoliation and renewal of cells at this age

It is now generally accepted that sediment counts of more than 100 cells per mm³ are highly suggestive of renal disease Counts of 20–100 cells per mm³ induce suspicion of a pathological condition and necessitate repeated examinations. Count of 20 cells per mm³ or less are considered normal (0, 7 10). These limits do not apply to the sediment counts in the urines of neonates In the present study more than 20 cells per mm³ were found in the major ity of specimens examined. One hundred and thirty nine specimens contained more than 50 cells per mm³ and of the 380 specimens examined 43 contained more than 100 cells per mm³ Only seven count exceeded 200 cells per mm³ The infant with extraordinarily high sediment count were followed closely for 10 to 15 days. All counts fell to average values during this time and it was not possible to demonstrate pathological traits in any of the infants. There is evidence however that both intrauterine asphyxia and perinatal asphyxia may induce transient renal damage with increased cell excretion into the urine (5 8) It is possible that a milder degree of hypoxia of some duration ha a similar effect on the fetal kidney Conditions with a moderate fetal hypoxia may be concealed during the pregnancy The highest sediment counts in the present study suggest that some intrauterine renal damage occurred in these infant Uric acid infarctions might possibly play a part although no correlation could be found between the amounts of organic and inorganic sediment in the present study

The sediment count are usually t the highest levels in the urines formed shortly

after birth. There is a fast decline in number of cells and casts excreted during the first 6 to 10 days of life and after this period the number of cells found is similar to that reported in studies of older infants and children. Normal birth is probably accompanied by some shock reaction in the infant s organs. Theoretically transient hypoxemia of the renal tissue caused by the birth process may be an important mechanism in the high sediment counts. In the present study there was no relation between the sediment counts and the duration of labour No comparisons of the oxygen saturation of the infant s blood during and after delivery have been performed and thus no certain conclusion can be reached in this regard It is equally tempting to suggest that the increased cell excretion is more the result of rapid tissue renewal in the tubule linings rather than changes induced by the shock of being born. The low content of cells found in the preformed urines is probably the result of intravesicular cell lysis in the d luted fetal urine

Three infants delivered by cesarean section before labour had started were followed, although omitted from the material of normal infants The sediment counts of these infants were all close to the verage found in the present m terial

The ranges of the normal Addis count for children and adults by earlier investigators (1 4 10 14) are too narrow for newborn infants. In addition to being cumbersome to perform, the Addis count suffers from great inaccuracies due to sampling difficulties in infants and is not to be relied upon. The method of expressing the sediment excretion in cells per cubic millimetre is more reliable. A combination of the two gives the best information of the sediment quantities. The standards given for newborn infants in the present study will provide a better foundation for comparison of such sediments in health and disease

Summary

Urine sediment counts were made in 380 specimens collected from 114 male and 27 female infants during the first 10 days after birth. In some the Addis counts were calculated. This method is considered inaccurate and erroneous in infants, however and it is advocated that the findings should be expressed in cells per cubic millimetre The counts found in the normal newborn infants are tabulated and the results are discussed. The range of normal sediment counts found exceeds that set for older children and adults in earlier publications.

Ref rences

1 Addis T A clinical lassification of Bright disease J A M A 85 163 19_5
2. Barrow P B The case against he ca better Amer J Med 44 1 1938
3. Craig, W S Urinary disorders occurring in the neona al perio l A h Dis hild 10 337 1935
4. Ogle, M. D The Addis count in be prog nosis of acut ne phri is in hildhood Arch Dis h ld J 23_ 1947
5 H ssman and A h Heers some of the urine of asphyxiated nd post-mature infants (to be published)
6 Houghton B J and Pears M A Cell excretion in normal urine Brit Med J I 6_2, 1957
7 James, U Urinary infection in the new born, Lancet I 1001 1959
8 Jonsson B Lower nephron nephrosis in asphyxia neonatorum Acta Pediat, 40 401 1951
9 Lippman R. W Urine and the Urinary

Sediment. A Practical Manual and Atlas, p. 5. C. C. Thomas, Springfield 1957

10. LYTTLE, J.: The Addis sediment count in normal children. *J Clin Invest 12* 87 1933.

11 REUSS, A. R. von: Die Krankheiten des Neugeborenen, S. 76. Julius Springer Berlin 1914.

12. SALOMONSEN L.: Propedeutisk pediatri, p. 149 J Grundt Tanum, Oslo 1952

13. SCHREINER, G E.: The identification and clinical significance of casts. *Arch Int Med 99* 356, 1957

14 SMITH, C. A.: The Physiology of the New born Infant p. 229. C. C. Thomas, Springfield, Ill. 1959

15. STOKE, A. W.: The normal Addis sediment count in children. *J Pediat, 12* 473, 1938.

16. STANSFELD J M.: Personal communication 1960.

17 STANSFELD J M and WEBB, J K. G.: Observations on pyuria in children. *Arch Dis Child 28* 386, 1953.

18. STERNHEIMER, R. and MALBIN B.: Clinical recognition of pyelonephritis, with new stain for urinary sediments. *Amer J Med, 11:* 312, 1951

19 THOMSON J.: Observations on the urine of the new-born infant. *Arch Dis Child, 19* 169 1944.

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Serum total and L(+)-Tartaric-acid inhibited Acid phosphatase Activity in Various Diseases in Childhood[1]

by ZVI LARON and AVIVAH KOWADLO

In 1937 Gutman & Gutman demonstrated the presence of acid phosphatase in human serum and found increased activity in patients with metastasing prostatic carcinoma. Since then occasional increased activity has been reported in adults in other diseases, such as Paget's disease (13) hyperparathyroidism (10) osteoporosis (8) liver disease (3, 6) Gaucher's disease (23) and gonadal dysgenesis (7). In children in whom the normal serum acid phosphatase activity is higher than in adults (18) increased serum activity of this enzyme has been reported in rare instances of osteopetrosis (24) osteogenesis imperfecta (10, 1) Niemann-Pick's disease (14) and more recently in Gaucher's disease (5).

In the present investigation serum total and tartaric-acid-inhibited acid phosphatase activities have been measured in children suffering from a variety of diseases with the aim of defining under which conditions the activity of these enzymes is altered in childhood.

Subjects and Methods

Children from the Pediatric Department and Pediatric Metabolic and Endocrine Clinic were studied. Blood was taken from the antecubital vein and the serum was separated within one to two hours. Hemolyzed samples were discarded. Acid phosphatase activity was determined immediately or after storage in the deep freeze for periods not longer than 3 days. According to our experience, storage did not alter the enzyme activity.

Serum total acid phosphatase (TAP) was determined by the method of King (15) slightly modified as described previously (16). L(+)-tartaric-acid-inhibited acid phosphatase (TIAP) was determined according to *Fishman & Lerner* (9). The mean normal values obtained in our laboratory for TAP are: age 1 year 5.22 KAU ±1.26 (SD); 1–5 years 4.63 KAU ±0.93 (SD); 5–13 years, 4.92 KAU ±0.93 (SD); TIAP for all ages, 0.21 KAU ±0.28 (SD). Alkaline phosphatase was measured in Bodansky units (2).

Results

The overall results of the TAP and TIAP measurements obtained are graphically shown in Figs. 1 and 2 and analysed for the various diseases in Tables 1–4.

Rheumatic fever

Twenty four children suffering from acute rheumatic fever were studied either before or during the first days of treatment (Table 1). With one exception the laboratory data in all cases pointed to

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Sediment. A Practical Manual and Atlas, p. 25. C. C. Thomas, Springfield 1957

10 Lyttle, J.: The Addis sediment count in normal children. J Clin Invest 12 87 1933.

11 Reuss, A. R. von: Die Krankheiten des Neugeborenen, S. 26 Julius Springer Berlin 1914

12. Salomonsen L. Propedeutisk pediatri, p. 149 J Grundt Tanum, Oslo 1952.

13. Schreiner, G E.: The identification and clinical significance of casts. Arch Int Med 99 356, 1957

14. Smith C. A. The Physiology of the New born Infant p. 229 C. C. Thomas, Springfield, Ill. 1959

15 Snoke, A. W The normal Addis sediment count in children. J Pediat, 12 473, 1938.

16. Stansfeld, J M.: Personal communication 1960.

17 Stansfeld J M. and Webb, J K. G.: Observations on pyuria in children. Arch Dis Child, 28 386, 1953.

18. Sternheimer, R. and Malbin B.: Clinical recognition of pyelonephritis, with new stain for urinary sediments. Amer J Med 11 312, 1951

19 Thomson J. Observations on the urine of the newborn infant. Arch Dis Child, 19 169 1944.

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TABLE .. *Serum acid phosphatase activity in children with bone disease.*

Patient	Age years	Sex	Diagnosis	Total acid phosphatase KA units	Tartaric-acid-inhibited acid-phosphatase KA units	Alkaline phosphatase Bodansky units
1. C.B.	2/12	M	Rickets	11.3	1 1	21.4
2. A.C.	3/12	M	Rickets	4.3	0.3	—
3. Z.C.	4/12	M	Rickets	8.1	0.2	29.6
4. S.G.	4/12	F	Rickets	3.9	0.3	18.4 24
5. T.A.	5/12	F	Rickets	9 7	—	14.8 15
6. I.S.	5/12	M	Rickets	5	0.1	16.7 24.6
7 L.G.	7/12	M	Rickets	3.3	0	10.8
8. S.J	10/1	M	Rickets (healed)	5.6	—	4
9. A.L.	11/12	M	Rickets	2.8	—	31.6
10. M.N	1/365	F	Hypocalcemia	5 1	0	8.2
11 M.R.	3/12	F	Osteogenesis imp.	7.2	2.4	—
12. D.B.	14/12	M	Familial dyschondroplasia	5	—	—
13. B.P	6	F	Multiple malformations of spine	6.0	0	—
14. M.H.	8	F	Dyschondroplasia	8.5 3.1	1.8	9
15. B.G.	9	M	Bone cyst	3.5	—	5
16. C.O.	10	F	Mult.cong osseous malformations	4.6	—	12.0
17 N.S.	11	M	Osteophytes	5.4	0.3	—

Bone diseases

In nine infants with rickets (Table 9) serum TAP activity was markedly increased in two and lower than 1 SD below the mean in three cases being within the range of mean ± 1 SD in the remaining TIAP determined in six infants was abnormally elevated in only one instance

Serum alkaline-phosphatase activity was elevated in six of the children, but there was no correlation with any of the acid phosphatases. Among other bone diseases, one 3-month-old infant with osteogenesis imperfecta exhibited increased activity of both TAP and TIAP

TAP as well as TIAP have been determined also in various other diseases as shown in the Figures 1 and ..

Discussion

Acid phosphatases are widely distributed enzymes (25) but our knowledge is scant concerning their metabolic role in normal and pathological states (20)

Using different substrates and inhibitors, an attempt was made to differentiate several acid phosphatases. the prostatic phosphatase is inhibited by L(+)-tartaric-acid (21) erythrocyte acid phosphatase is inhibited by formaldehyde (6)

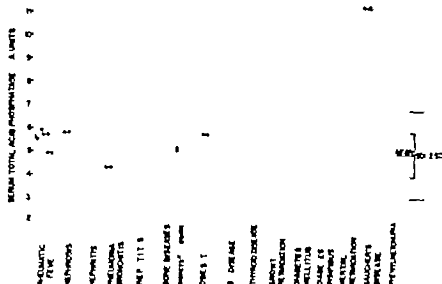


Fig 1 Serum-total acid phosphatase activity in variety of diseases in childhood. The normal mean is applicable to all groups besides "rickets" Since this disease occurs in infant under the age of one year the normal mean is slightly higher



Fig — Serum L(+)-tartaric-acid-inhibited acid phosphatase in a variety of diseases in childhood.

and cupric ions (22) and the acid phosphatase of the serum from patients with Gaucher's disease is inhibited mainly by fluoride (5) The last mentioned phosphatase also splits only disodium phenyl phosphate substrate but not beta-glycerol phosphate Certain studies make the above classification questionable: for example tartaric-acid inhibited acid phosphatase ha been found in tissues other than the prostate and in the serum of both human and animal females (0 17 18). The present study also bears on these facts.

During the process of growth, infants and children have a higher serum acid phosphatase activity than adult (14). This was demonstrated by using a substrate disodium phenyl phosphat but wa not evident when beta glycerophosphate wa used (11)

Only occasional report have been made of increased serum acid phosphatase activity in children. These observations con

cern few cases of osteopetrosis and osteogenesis imperfecta (*4, 10) Recently a rise of serum total acid phosphatase has been observed in Gaucher's disease (5). No mention of acid phosphatase activity decreasing below normal range is made in any of the reports.

In the present investigation it was noted that the variation of serum total acid phosphatase in the conditions listed below was greater than that found in normal children, i.e. in acute rheumatic fever diseases of the lungs, kidneys and bones. Occasional increased activity in kidney and bone disease has been encountered in adults (6 8 13). A most interesting finding was the high incidence of increased total and tartaric-acid-inhibited acid phosphatase in children with acute rheumatic fever Although no explanation for these observations is at hand at the present time, it seems that the phosphatase activity is linked to the disease process. The fact that the high acid phosphatase activity decreased in a few instances during clinical improvement supports this assumption. Moreover the decrease of enzyme activity occurred with either salicylate or prednisone therapy

Although slightly decreased activity was observed in many of the disorders studied, marked decrease in activity of serum acid phosphatase was observed in some cases of growth retardation, nephritis and one patient with rickets.

The source of the serum phosphatases in both normal state and pathologic conditions is not yet clear It is worth mentioning that acid phosphatase is also present in other body fluids and in increased activity of this enzyme in CSF has been found in certain neurological disorders, both in adults (4) and in children (19). Pleural and ascitic fluid in children were also found to contain increased activities of acid phosphatases under certain circumstances (20)

As in previous observations (16) no correlation between serum acid and alkaline phosphatase was found even in those patients who suffering from rickets, had high activity of the alkaline phosphatase

The question why only a certain proportion of cases of a particular disease show a deviation from the normal range of activity of serum acid phosphatases remains unanswered until more data are accumulated and better knowledge concerning the metabolic role of these enzymes is available.

Summary

Serum total acid phosphatase (TAP) and L(+)-tartaric-acid-inhibited acid phosphatase (TIAP) activities have been determined in 95 and 55 children respectively suffering from a variety of diseases. It was found that in a certain number of children with rheumatic fever diseases of the liver lungs, kidneys and bones the variations in total-serum acid phosphatase were considerably greater than in normal children. The activity of TIAP was *significantly* increased in rheumatic fever

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facilitated by comparing the synchronously recorded leads. Where the length of the P wave, QRS complex or T wave varied from lead to lead it was measured in that lead in which it was longest. When the length of the QT interval varied an average value was calculated. The measured QT interval was corrected to compensate for the influence of heart rate by the application of Bazett's equation (4) and the term QTc used to designate the resulting value; this calculation was made by slide-rule.

The length of the ST segment was obtained by subtracting the sum of the QRS complex and the T wave, measured in the lead in which they were longest, from the average QT interval. Thus subsequent calculations were based on the shortest ST segment as recommended by Ashman & Hull (3).

The range, arithmetic mean and standard deviation of the mean (S.D.) of the heart rate and intervals were calculated for each 3-month period and for the entire sample. Correlation coefficients (r) were calculated for the P wave, PR interval, QRS complex, T wave, QT interval and ST segment against heart rate (beats/minute) and age (weeks). These were tested for significance.

Results and Discussion

Heart Rate

The lowest and highest heart rates were 102 per minute at the age of 40 weeks and 207 per minute at the age of 2 weeks. The mean rate for the entire sample was 141 per minute, but in the fourth quarter the mean rate was 20 beats per minute less than in the first quarter (Table 1). An unexpected finding was that the mean rate in the third quarter was less than in the fourth. Nevertheless a highly significant negative correlation ($p < 0.01$) of heart rate with age was disclosed ($r = -0.2951$).

In 47 subjects the heart rate was between 120 and 130 per minute and in 80 between 100 and 160 per minute. Of the 20 subjects whose heart rate was 160 or over, 11 were less than 3 months old and 6 were between 3 and 6 months old.

It is obvious that these heart rates cannot be regarded as basal or resting values because some subjects were excited by the disturbance inherent in the recording procedure. Nevertheless, as Ziegler (23) has pointed out, such measurements give a useful indication of the wide range through which the heart rate may vary in health. No one would deny that the heart rate should be counted during sleep but in infancy this is not always practicable. The practice at this hospital is to make the count by auscultation rather than by palpation of a peripheral pulse, which is not only less accurate but sometimes impossible. The disadvantage of auscultation is that the baby is often awake and restless by the time the praecordium is exposed.

TABLE 1. *Heart rate (beats per minute) and mean values of cardiac cycle (second)*

Age group	No. of observations	Range of observations	Arithmetic mean	S.D.	Arithmetic mean of cardiac cycle
2 weeks–3 months	25	118–207	153.6	22.3	0.39
3–6 months	25	118–200	143.2	1 4	0.42
6–9 months	25	115–182	130.5	13.52	0.46
9–12 months	25	102–200	135.6	22.59	0.44
2 weeks–12 months	100	102–207	141.2	22.31	0.4

TABLE 2 *P wave (seconds)*

Age group	No. of observations	Range of observations	Arithmetic mean	S.D.
2 weeks–3 months	45	0.04–0.07	0.058	0.0060
3–6 months	25	0.04–0.08	0.064	0.0071
6–9 months	25	0.06–0.08	0.066	0.0057
9–12 months	5	0.06–0.08	0.069	0.0122
2 weeks–12 months	100	0.04–0.08	0.063	0.0061

Published figures for the heart rate of healthy infants show wide differences but a feature common to most is a declining rate with increasing age

Arrhythmia

Sinus arrhythmia was the only disturbance of rhythm encountered. The diagnosis, based on the criterion proposed by Krumbhaar & Jenks (14) was made only if the greatest difference between cardiac cycles was 0.1 second or more Five examples were found. The subjects were 4 7 9 10 and 11 months old, and had average rates of 128, 120 105, 110 and 123 per minute, respectively The infrequency of sinus arrhythmia in this sample lends support to the opinion of several authors, including Lincoln & Nicolson (15) that this arrhythmia is less common in infancy than in later childhood and that its incidence rises as the heart rate falls.

The infant with sinus arrhythmia at the age of 10 months had been examined 11 weeks before. In the earlier record the difference of 0.04 second between cardiac cycles was insufficient to permit a diagnosis of sinus arrhythmia. There was little difference between the average heart rates of the two records (115 in the first 110 in the second), and this suggests that slowing of the pacemaker is not the sole factor causing sinus arrhythmia.

A difference between cardiac cycles of less than 0.1 second was found in 69 electrocardiograms, but unlike true sinus arrhythmia the incidence was not substantially greater in older infants. There were 17 examples in the first quarter 16 in the second, 19 in the third and 17 in the fourth.

P wave

The duration of the P wave ranged from 0.04 to 0.08 second, and the mean values increased with age (Table 2). A highly significant positive correlation was found between the length of the wave and age ($r = +0.5045$) This lengthening of the P wave with increasing age accords with Ziegler's findings (23) but his mean values are as much as 0.012 second less. The range of values encountered in the present series is precisely the same as that quoted for the whole of childhood by Hafkesbring *et al* (12) There was no significant correlation between the length of the P wave and the heart rate ($r = -0.1003$) It seems probable that the increasing width of the P wave observed during the first year of life reflects the increasing mass of the atria.

TABLE 3. *PR interval (seconds)*

Age group	No. of observations	Range of observations	Arithmetic mean	S.D.
weeks–3 months	[illegible]5	0.08–0.14	0.099	0.01[illegible]7
3–6 month	25	0.08–0.12	0.102	0.0111
6–9 months	25	0.09–0.14	0.111	0.0133
9–12 months	[illegible]5	0.08–0.12	0.103	0.017
2 weeks–1 months	100	0.08–0.14	0.104	0.0124

PR interval

The average duration of the PR interval for the sample was 0 104 second and the range of individual values was from 0 08 to 0 14 second (Table 3) The mean PR interval lengthened from the first to the third quarter but was less in the fourth quarter than in the third. This suggested that the PR interval might be more related to heart rate than to age because as already noted, the mean heart rate was greater in the fourth than in the third quarter There was a significant correlation ($p = < 0.05$) between the length of the PR interval and both heart rate ($r = -0.230*$) and age ($r = +0.2034$) but the influence of the heart rate was the greater as indicated by the linear regression equation of PR on age and heart rate viz.— PR = 0 000148310 Age (weeks) – 0 00011 ?10 Heart Rate (beats per minute) – 0 11 111 Thus the PR interval tends to lengthen both with decreasing heart rate and with increasing age Alimurung & Massell (*) came to the same conclusion but Savilahti (19) and others have doubted whether the PR interval is influenced by heart rate The mean values obtained for the length of the PR interval are lower than those given by Burnett & Taylor (5) and by Epstein (8), but agree closely with those of Ziegler (23).

The ventricular compl xes

The length of the QRS complex in the entire sample varied from 0.0[illegible] to 0.08 second and the mean value wa 0 062 second (Table 4) The mean values increased progressively from the first to the fourth quarter Highly significant correlations ($p = < 0.01$) existed between the length of the QRS complex and both age ($r = +0.5287$) and heart rate ($r = -0.43[illegible]1$) With decreasing heart rat or increasing age the QRS complex lengthens and this finding is in agreement with that

TABLE 4 *QRS complex (second)*

Age group	No. of observations	Range of observations	Arithmetic mean	S.D.
weeks 3 months	5	0.03–0.0[illegible]	0.0[illegible]	[illegible]
3–6 months	[illegible]	0.03–0.07	0.059	[illegible]
6–9 months	2[illegible]	0.03–0.0[illegible]	0.064	[illegible]
9–1 month		0.06–0.08	0.06[illegible]	0.0042
weeks 1 month	1	0.0[illegible]–0.08	0.062	[illegible]

TABLE 5. *T wave (seconds)*

Age group	No. of observations	Range of observations	Arithmetic mean	S.D
2 weeks–3 months	25	0.09–0.14	0.111	0.0134
3–6 months	25	0.10–0.13	0.115	0.0129
6–9 months	25	0.11–0.15	0.126	0.0112
9–12 months	25	0.11–0.15	0.129	0.0133
2 weeks–12 months	100	0.09–0.15	0.120	0.0144

of Savilahti (8) The mean values given by Ziegler (23) are higher and do not show the same gradual lengthening with age. On the other hand, Seham (21) and Burnett & Taylor (5) reported values which were much lower and Seham's are so low that it seems possible that he may have ignored shallow Q waves when making his measurements. The present figures are however closely similar to those of Jundell & Stenström (13).

The length of the T wave lay between 0.09 and 0.15 second, and the average value for the whole sample was 0.120 second (Table 5). The mean values increased progressively from the first to the fourth quarter This trend is in agreement with the findings of Ziegler (23) but his mean values are much greater A highly significant degree of correlation (p = < 0.01) existed between the duration of the T wave and both age (= + 0.3288) and heart rat (= − 0 1 4) but the influence of rate was by far the greater With decreasing heart rate or increasing age the T wave lengthens.

Both the QRS complex and the T wave lengthen throughout the first year of life, and it seems likely that this is a reflection of the growth of the ventricles.

QT interval

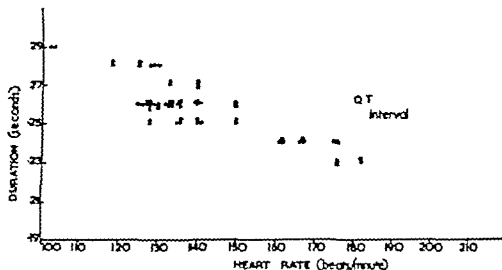
The QT interval or electrical systole includes the QRS complex, the ST segment and the T wave.

The range through which the QT interval varied during infancy was found to be 0.21 to 0.29 second with a mean value of 0.257 second (Table 6)

Bazett (4) recognised that the length of of the QT interval was affected by the heart rate, and in the belief that the relationship could be expressed by a straight line graph, he introduced a formula to express such an association. Some investigators have suggested that the rela

TABLE 6 *QT interval (seconds)*

Age group	No. of observations	Range of observations	Arithmetic mean	S.D
2 weeks–3 months	25	0.21–0.28	0.250	0.0134
3–6 months	25	0.21–0.29	0.252	0.0141
6–9 months	25	0.22–0.29	0.264	0.0150
9–12 months	25	0.23–0.29	0.264	0.0175
weeks–1 months	100	0.21–0.29	0.257	0.0174



Scatter diagram relating 100 measurements of the QT interval t the prevailing heart rat

tionship is not linear and have devised other methods for relating the measured QT interval to the heart rate (3 9 10 20)

The individual measurements of the QT interval were plotted against the heart rate and it was obvious that the QT interval lengthened as the heart rate fell (Figure) This is shown statistically by a very significant negative correlation (r = − 0.85") A significant positive correla tion existed between the QT interval and age (r = + 0.3275) but the influence of age was much less than that of rate as shown by the linear regression of QT on age and heart rate viz 0 0001043 03 Age (weeks) − 0 00064 9885 Heart Rate (beats per minute) − 0 347013

The range of QTc values for the whole period under review was 0.36 to 0 4.., with an average of 0.39" (Table 7). A survey of the findings of other investigators discloses appreciable differences among their mean values. Epstein (8) accepted a value of 0.38 Allmurung *et al* (1) and Yu, Joos & Katsampes (22) accepted a value of 0 404 Ziegler (23) found mean values at various ages from one week to one year t lie between 0.385 and 0 408 and the scatter of values was greater than in the present series in which 0% of all values are within the range mean ± 1 × S D and 9" , within the range mean ± × S.D

TABLE 7 *QTc*

Age group	No. of observations	Range of observations	Arithmetic mean	S.D.
week 3 m hs	5	0.370–0.415	0.379	0 0116
3–6 mon h	.3	0.345–0 410	0 355	0 0119
6–9 mon h		0.360–0.415	0.390	0.01.1
9–1 mon hs	25	0.375–0.4?0	0.393	0.0124
week 1 mon hs	1)	0.360–0.450	0 393	0 0133

TABLE 8. *ST segment (seconds)*

Age group	No. of observations	Range of observations	Arithmetic mean	S.D
2 weeks–3 months	25	0.06–0.11	0.081	0.0153
3–6 months	25	0.06–0.10	0.078	0.0116
6–9 months	25	0.05–0.10	0.074	0.0156
9–12 months	25	0.05–0.08	0.065	0.0082
2 weeks–12 months	100	0.05–0.11	0.075	0.0127

ST segment

The length of the ST segment was examined because this portion of the QT interval appears to be particularly susceptible to changes in the concentration of calcium in the body fluids. It seemed possible that measurement of the ST segment might permit the recognition of significant changes in its length too small to lengthen or shorten the entire QT interval to an appreciable extent.

The ST segment was found to vary between 0.05 and 0.11 second with a mean value of 0.075. It was observed from the mean values for the four quarters that this interval shortened with increasing age (Table 8). Highly significant negative correlations ($p = < 0.01$) existed between the length of the segment and both age ($r = -0.3554$) and heart rate ($r = -0.2883$). In other words, the ST segment tends to shorten both with increasing age and heart rate.

Thus in this sample both the QRS complex and the T wave lengthened as age increased but the ST segment shortened. This finding was supported from a comparison of the components of the QT interval of the 10 subjects twice examined. In the later records the QRS complex had lengthened or remained unchanged in 10 subjects, the T wave in 7 but the ST segment had shortened or remained unchanged in 8.

Summary and Conclusions

The standard limb leads of the electrocardiograms of 100 healthy infants were examined. The heart rate and the length of the P wave, PR interval, QRS complex, T wave, ST segment, QT interval and the corrected QT interval (QTc) were measured. The heart rate showed wide variations throughout the period under review but decreased as age advanced. Sinus arrhythmia was encountered only five times but minor differences in the length of cardiac cycles occurred frequently. No other disturbance of rhythm was observed. The P wave, PR interval, QRS complex and T wave lengthened as age increased and, the P wave excepted, as the heart rate slowed. The ST segment tended to shorten both with increasing age and increasing heart rate. The linear association between the length of the QT interval and the heart rate was confirmed. The observed values for the corrected QT interval (QTc) fell within the range 0.36 to 0 4–

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References

1 Alimurung M M, Joseph L G Crause, E. and Massell, B. F The Q-T interval in normal infants and children. *Circulation, 1* 1379 1950.

2. Alimurung, M. M and Massell, B. F.: The normal P R interval in infants and children *Circulation, 13* ..37 1956.

3. Ashman R. and Hull, E.: Essentials of Electrocardiography 2nd ed. The MacMillan Co., New York, p. 139 14., 1945.

4 Bazett H C An analysis of the time-relations of electrocardiograms. *Heart 7* 353, 1920

5. Burnett C T and Taylor, E. L.: Electrocardiograms on 167 average healthy infants and children. *Amer Heart J 11* 185, 1936

6. Coleman E. N Electrocardiographic changes in idiopathic hypercalcaemia of infancy *Brit Med J 2* 467 1959

7. Engel, F Das Elektrokardiogramm des gesunden Frühgeborenen, Neugeborenen und Säuglings. *Z Kinderhk 59* 350 1937-38

8. Epstein N The heart in normal infants and children. *J Pediat 32* 39 1948.

9. Fridericia, L. S Die Systolendauer im Elektrokardiogramm bei normalen Menschen und bei Herzkranken. *Acta Med Scand 53* 469 468 1920

10. Funaro, N and Nicolai G Das Elektrokardiogramm des Säuglings. *Zbl Physiol, 2* 58, 1908

11 Groedel, F M and Miller, M. Electrocardiographic studies in the newborn. *Exp Med Surg, 2* 110 1944

12. Hafkesbring Ellen M Drawe, C Ebert E and A... R., Children electrocardiograms 1 Measurements for 100 normal children. *Amer J Dis Child* ..3 14..7 1937

13. Jundell, I and Stenström N.: A study of the electrocardiogram in infants of normal conditions and during treatment with cod liver oil and irradiation. *Acta Pædiat, 1* 112, 1921

14 Krumbhaar, E B. and Jenks, H. H: Electrocardiographic studies in normal infants and children. *Heart, 6* 189, 1915.

15. Lincoln E. M and Nicolson G H. B The hearts of normal children. III. Electrocardiographic records. *Amer J Dis Child 35* 1001 1928.

16. Mannheimer, E.: Calibrated Phonocardiography and Electrocardiography *Acta Pædiat 28* 8 ppl. 44 1940

17 Nadrai, A.: Das Elektrokardiogramm im Säuglingsalter *Z Kinderhk 60* 5 193

18. Ravilanti, M.: Concerning the effect of frequency and age in the QRS time of the electrocardiogram. *Acta Med Scand, 123* 143, 1946.

19. Ravilanti, M.: On the normal and the pathological PQ time of the electrocardiogram. *Acta Med Scand 123* ... 1946.

20. Schlamowitz, I An analysis of the time relationships within the cardiac cycle in electrocardiograms of normal men. I. The duration of the QT interval and its relationship to the cycle length (R R interval). *Amer Heart J 31* 329 1946

21 Ziegler, M.: Electrocardiography in children. Abt Pediatrics Vol II. W B Saunders Co Philadelphia, p. ... 19 ...

22. ... P N G Joos, H A. and K... C P ... polar electrocardiograms in normal infants and children. *Amer Heart J 41* 91 1951

23. Ziegler, R. F Electrocardiographic Studies in Normal Infants and Children. Charles C Thomas, Springfield, Ill 1951

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Fractional Collection of Urine in Very Small or Ill Infants

by R. L. CORT

Many methods of collecting urine in infants have been described Each has certain advantages, the most important of which is probably the familiarity of the nursing and medical staff with a given technique.

The method to be described was worked out to fulfil certain requirements: simplicity so that it can be carried out by nurses with other routine ward duties, measurement of very small quantities of urine at relatively short intervals and absence of danger of skin or other trauma, particularly in critically ill infants.

Its chief use has been in healthy and ill newborn premature infants 0–5 days of age although full-term infant 0–?4 hours of age have also been studied. No provision for the collection of stools (meconium) is made.

Method

For collection of urine samples, the equipment shown in Fig 1 is used It consist of

1 A pad of washed bsorbent cotton. The cotton is weighed, washed with 0.1 N sulphuric acid, then with distilled water and then suction- and heat-dried A weight of 3 g was chosen for convenience This amount of cotton will absorb abou 30 ml of urine without leakage

2. A thin rubber cap int which he cotton fits.

3. A pair of simple holding panties with four tie fastenings, which permit examination of the cotton, cleaning rectal temperature measurement etc., with minimal disturbance of the infant.

4. A numbered glass weighing bottle containing 20–25 ml of water or other eluting fluid.

The washed cotton is fitted into the rubber cap and placed on the infant perineum (Fig 2) With either sex the cotton is hollowed out to admit the penis or the labia. If avoidance of contamination with meconium is absolutely necessary a small strip of non-irritant adhesive tape may be used t fix the posterior margin of the cap to the perineal body In practice, contamination with stools is uncommon or very slight.

A folded diaper is placed over the cap and through the infant's legs, and held in place by the panties. Thus, slight pressure is exerted t hold the cotton in place Newborn premature infants, particularly ill ones, move very little, and our newborn infant are swaddled, so that displacement of the cotton wad through the infant activity is rare

Every three hours, t feeding time the nurse unties the panties and lifts the upper edge of the cap t see if urine has been passed If the cotton pad is wet, she removes it and places it in a weighing bottle the number of which is noted The infant may be weighed with the collecting cotton in place.

For estimating the urinary constituents the bottle is weighed to determine the volume of urine the cotton thoroughly

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References

1 Alimurun M. M Joseph, L. G Craige, E. nd M. Small, B F.: The Q T interval in normal infant nd children. C rcula t on J 1329 1950.
2. Alimurun M. M. and Massell, B. F.: The normal P R interval in infants and children. C rculation, 13 57 1956.
3. Ashman R. nd Hull, E. Essentials of Electrocardiography 2nd ed The MacMillan Co New York, p. 139 16, 1945.
4 Barett H C.: An analysis f the time relations of electrocardiograms. Heart 7 353, 1920.
5 Burnett C T and Taylor, E. L.: Electrocardiograms on 167 verage healthy infa t nd hildren. Amer Heart J 11 185, 1936
6. Coleman E. N Electrocardiographic hanges in idiopathic hypercalcaemia of infancy Brit Med J 2 467 1959
F L. E.: Das Elektrokardiogramm des gesunden Fruhgeborenen, Neugeborenen und Säuglings. Z Kinderhk 59 3 9 193 38
8. Epstein N The heart in normal infants and children. J Pediat 32 39 1948.
9 Fridericia L. S Die Systolendauer im Elektrokardiogramm bei normalen Menschen und bei Herzkranken. Acta Med Scand 53 469, 489, 1920.
10 Funaro, N nd Nicolai, G Das Elektrokardiogramm des Säuglings. Zbl Physiol 58, 1908.
11 Grossel, F M nd Miller, M Electrocardiographic studies in the newborn. Exp Med Surg 110, 1944
1 Hayek M Elea or M. Drawe Cather E. and A R.: Children lectrocardiograms I Measurements for 100 normal hildren. Amer J Dis Child 3 1437 1937
13. Jundell, I and Stenström N.: A study of the electrocardiogram in infants of normal conditions and during treatment with cod liver oil and gosterol Acta Pediat 12 113, 1931
14 Krumbhaar, E. B. and Jenks, H. H.: Electrocardiographic studies in normal infants and hildren. Heart, 6 189 1915.
15. Lincoln E. M. and Nicholson G. H B The heart of normal children. III. Electrocardiographic records. Amer J Dis Child, 35 1001 1928.
16. Mannheimer, E.: Calibrated Phonocardiography and Electrocardiography Acta Pediat 1 Suppl. 44 1940.
17 Nadrai, A.: Die Elektrokardiographie im Säuglingsalter Z K nderhk, 60 3 193
18. Savilahti, M.: Concerning the effect of frequency and age in the QRS time of the electrocardiogram. Acta Med Scand, 123 143, 1946.
19 Savilahti, M.: On the normal and the pathological PQ time of the electrocardiogram. Acta M d Scand 123 2, 1946.
20. Schlamowitz, I. An analysis of the time relationships within the cardiac cycle in electrocardiograms of normal men. I. The duration of the QT interval and its relationship t the cycle length (R R interval) Amer Heart J 31 329, 1946
1 Sehaw, M.: Electrocardiograph in children Abt Pediatrics Vol. II W B Saunders Co Philadelphia, p 2 , 19
22 Yu P N G., Joos, H. A. and K rs C. P Unipolar electrocard grams in normal infant and hildren. Amer Heart J 41 91 1951
23. Ziegler, R F Electrocardiographic Studies in Normal Infant and Children. Charles C Thomas, Springfield, Ill 19 1

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Clinodactyly and Brachymesophalangia of the Fifth Finger

by A. F. ROCHE

The term clinodactyly is applied to a curvature of the extended finger and in the case of the fifth finger this curvature is always towards the fourth. A slight curving in this direction is normal and earlier workers have restricted the use of this term to cases of marked inclination, determined on a basis of subjective judgement. By brachymesophalangia is meant a shortness of the shaft of the middle phalanx. Schmid & Junker (10) have suggested a definition of brachymesophalangia based on measurements of phalangeal shaft length. Other workers have subjectively assessed the length of the middle phalangeal shaft relative to the lengths of the other phalangeal shafts in the digit and the size of the hand and have used their judgement to determine the amount of shortness necessary for the application of this term. During this investigation similar subjective methods have been followed before the term brachymesophalangia has been used.

Many have accepted without question the statement by Smith (11) that marked clinodactyly of the fifth finger is associated with a shortness of its middle phalanx, is present in nearly every mongoloid. The literature however contains few investigations of this alleged relationship in either mongoloid or normal children.

Review of Literature

Reports of the incidence of clinodactyly in mongoloids vary between 34.6% and [illegible] (Table 1) after the omission of the small series examined by West (15). The incidence of clinodactyly in normal children has been reported as 19.5% (15) and 5.2% [illegible] (Table 1). It is probable that much of this variation is due to the lack of a precise determination of normal limits and an application of terms based upon subjective criteria.

Clinodactyly could occur at either or at both the interphalangeal joints of the [illegible]. There is no information in the literature concerning the common site of clinodactyly except the observation of Thorsell [illegible] the clinodactyly of the fifth finger [illegible] occurred at the distal interphalangeal [illegible].

Brachymesophalangia of [illegible] has been reported in 62 of [illegible] (3) and in 10 of 700 normal [illegible] Hefke (3) reported clinodactyly [illegible] mongoloid in whom brachymesophalangia was present in the same [illegible] is higher incidence of clinodactyly [illegible] the general group of mongoloids [illegible] be concluded that these [illegible] associated. Opinions that [illegible] brachymesophalangia [illegible] bination have also been [illegible] for mongoloids and by [illegible] for normal children.

It has been stated that [illegible] creases with age in [illegible] though this is denied by [illegible] learned that brachymesophalangia [illegible] less marked in [illegible] age [illegible]

In the present study an attempt has been made to evaluate the possible association between clinodactyly and brachymesophalangia in the fifth fingers of both mongoloid and normal children.

Material and Methods

The selection of 192 mongoloid and 120 normal children has been described elsewhere (9). Observations have been made on serial dorso-palmar radiographs of the left hand of these children taken at regular intervals over a four year period. Only those radiographs have been utilized in which the fingers were extended fully and abducted slightly from each other. Clinodactyly and brachymesophalangia of the fifth finger have been identified in a mixed sequence of mongoloid and normal children by the same observer using subjective criteria similar to those of other workers.

Findings and Discussion

At all ages studied clinodactyly was more common in mongoloid than in normal children (Table 2). Combining both sexes and all ages the incidence was 55.2% for mongoloids and 32.5 for normal children. These findings are similar to those reported previously for mongoloids, but are higher than those reported previously for normal children (Table 1). Such observations do not justify the claim of Smith (11) that "this curve of the little finger occurs to a greater or less degree in nearly every case of mongol idiocy".

Clinodactyly occurred most commonly at the distal interphalangeal joint in mongoloids, which is in agreement with the observation of Thursfield (13). In normal children however it was slightly more common for both the proximal and distal interphalangeal joints to be involved (Table 2). This difference between the mongoloid and the normal children in the site of clinodactyly occurred even in those in whom the fifth middle phalanx was of the usual length and, consequently it could not have been dependent upon the marked difference between the mongoloid and normal children in the incidence of brachymesophalangia. In both mongoloid

TABLE 1 *The recorded incidence of clinodactyly*

Author	Number examined	Number with clinodactyly	% with clinodactyly
Mongoloid children			
West (1901)	9	1	11.1
Muir (1903)	[illegible]	9	34.6
Thursfield (1921)	[illegible]	12	50.0
Orel (1927)	26	33	[illegible]
Hefke (1940)	100	43	43
Benda (1949)			34.5
Engler (1949)			[illegible]
Oster (1953)	604	52	[illegible]
Levinson *et al.* (1955)	50	34	[illegible]
Normal children			
West (1901)	603	11[illegible]	1.5
Engler (194[illegible])	500	[illegible]	[illegible]

Not recorded by author

TABLE 2 *Males/Females*

Chronological age in years	Total children	Site of clinodactyly: Proximal inter phalangeal joint only	Distal inter phalangeal joint only	Both inter phalangeal joints	Percentage with clinodactyly	No. with brachymeso-phalangia
Mongoloid children						
0–2	7/9	0/0	4/4	0/0	57.1/44.4	2/5
2	46/46	2/2	14/19	5/1	45.7/4 .8	10/9
8+	37/47	4/4	12/28	6/1	59.5/70.2	11/14
Total	90/102	6/6	30/51	11/2	52.2/57.8	24/28
Normal children						
3–7	60/60	1/3	7/9	14/6	36.7/28.3	2/2

and normal children, clinodactyly at both the proximal and distal interphalangeal joints of an individual was more common in males than in females.

Brachymesophalangia was much more common in the mongoloid (27.1%) than in the normal children (3.3%) (Table 2). This is lower than the incidence reported for mongoloids by Hefke (3) but is similar to that reported for normal children by Stettner (12). No case of absence of the fifth middle phalanx was observed among either the mongoloid or the normal individuals.

When mongoloid and normal children of similar age were compared (Table 3) it was found that clinodactyly occurred more commonly in the mongoloid (47.2%) than in the normal children (32.5%). However clinodactyly associated with a middle phalanx of normal length was observed with almost equal frequency in both mongoloids (25.0%) and normal children (29.2%). In all children in whom brachymesophalangia was noted clinodactyly was present also with the exception of three mongoloids. The fact that these conditions sometimes occur independently is contrary to the report of Smith (11). Brachymesophalangia associated with clinodactyly was present in 22.2% of the mongoloid children under the age of 8 years but in only 3.3% of normal children of comparable age.

TABLE 3 *Findings in children aged less than 8 years*

Conditions	Mongoloid No.	Mongoloid %	Normal No.	Normal %
Clinodactyly without brachymesophalangia	27	25.0	35	29.2
Clinodactyly with brachymesophalangia	4	22.2	4	3.3
Brachymesophalangia without clinodactyly	3	2.8	—	—
Neither clinodactyly nor brachymesophalangia	54	50.0	81	67.5
Totals	108	100.0	120	100.0
Total clinodactyly	51	47.2	39	32.5
Total brachymesophalangia	27	25.0	4	3.3

The serial records of both mongoloid and normal children show a remarkable stability for each individual in both the site and the degree of clinodactyly and the extent of brachymesophalangia. However brachymesophalangia became slightly less common as older groups of mongoloids were considered These findings are in agreement with those of Øster (7) but are contrary to those of West (16) and Brousseau (1) concerning clinodactyly and those of Hefke (3) in regard to brachymesophalangia.

Conclusion

A consideration of the above findings leads one to conclude that there are two types of clinodactyly of the fifth finger This is best illustrated by reference to the findings on mongoloid and normal children of comparable age (Table 3) In some children with clinodactyly the fifth middle phalanx is of normal length. This type of clinodactyly occurs with approximately equal frequency in both mongoloid and normal children, although the common site of such clinodactyly is different in the two groups. In other children, clinodactyly of the fifth finger is associated with brachymesophalangia and this type is much more common in the mongoloid group (22.2%) than in the normal group (2 3 %)

There is a similar marked difference between the mongoloid (25 0%) and the normal groups (3 3) in the incidence of brachymesophalangia. The brachymesophalangia was combined with clinodactyly in all but three of these children. It is suggested that brachymesophalangia should be used in the diagnosis of suspected mongolism and that if this is done the recording of clinodactyly provides no additional information that is diagnostically useful

Summary

1 Serial radiographs of the hands of 19 mongoloid and 1?0 normal children extending over four years have been used to investigate the frequency of clinodactyly and brachymesophalangia in the fifth finger and their changes during growth.

2. The incidence of clinodactyly in the mongoloid children was similar to that recorded in the literature but the incidence in the normal children was higher than that recorded previously. Clinodactyly with a middle phalanx of the usual length occurred with almost equal frequency in both mongoloid and normal children. There was a marked difference between the two groups of children in the incidence of clinodactyly associated with brachymesophalangia

3 It is suggested that brachymesophalangia is a more important physical sign of mongolism than clinodactyly Considering children under the age of 8 years it was present in 25.0 of the mongoloid group and 3.3% of the normal group and was almost always associated with clinodactyly

4 The incidence of clinodactyly did not vary with age but brachymesophalangia was slightly less common among older mongoloid

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References

1 Brousseau K.: Mongolism. The Williams and Wilkins Co., Baltimore 1928.

2. Engler, M.: Mongolism. John Wright and Sons Ltd., Bristol, 1949

3. Hefke, H. W Roentgenologic study of anomalies of the hand in one hundred cases of mongolism. *Amer J Dis Child*, *60* 1319–1323, 1940.

4. Levinson, A Friedman A. and Stamps, F Variability of mongolism. *Pediatrics* *16* 43–54, 1955.

5. Muir, J An analysis of twenty-six cases of mongolism. *Arch Pediat*, *20* 161 169 1903.

6. Ohm, H.: Zur Klinik der mongoloiden Idiotie. *Z Kinderheilk*, *44* 449–472, 1927

7 Oster, J Mongolism. Danish Science Press Ltd., Copenhagen, 1953.

8. Pol, —: "Brachydaktylie "Klinodaktylie Hyperphalangie und ihre Grundlagen. *Virchow Arch Path Anat*, *229* 388–530 1921

9 Roche, A. F and Sunderland, S.: Melbourne University child growth study *Med J Aust*, *1* April 25, 559–562, 1959

10. Schmid, F and Junker, F Die Brachymesophalangie des Kleinfingers. *Z Kinderheilk* *84* 399–407 1960

11. Smith, T T A peculiarity in the shape of the hand in idiots of the "mongol type. *Pediatrics*, *2* 315–320, 1896

12. Stettner, E.: Über die Beziehungen der Ossifikation des Handskeletts zu Alter und Längenwachstum bei gesunden und kranken Kindern von der Geburt bis zur Pubertät. *Arch Kinderheilk*, *68* 439–466, 1921

13. Thursfield H. Notes on mongolism. *Brit J Child Dis*, *18*. 18–21, 1921

14. Warner, E. N.: A survey of mongolism with review of one hundred cases. *Canad Med Ass J* *33* 495–500, 1935.

15 West J P A note on the little finger of the mongolian idiot and of normal children *Arch Pediat*, *18* 918–924 1901

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Discussion on the Advisability of Performing Exchange Transfusion in Neonatal Jaundice of Unknown Aetiology[1]

by DYRE TROLLE

The indications for exchange transfusion in neonatal jaundice of unknown aetiology have been much discussed in recent years. These procedures are performed because it is known that some of the children will develop cerebral palsy as a consequence of the toxic effect of hyperbilirubinaemia on the nerve cells of the basal ganglia of the brain. Both the anatomical lesions and the clinical signs are the same as those seen in untreated newborn children with haemolytic disease due to Rhesus incompatibility. It is evident from the investigation of Plum (8) of 478 children more than one year of age suffering from cerebral palsy that there is an aetiological relationship between severe jaundice (with or without blood-group incompatibility) occurring in the first weeks of life and the subsequent development of athetosis. Other forms of cerebral palsy are probably not due to neonatal jaundice.

The exact bilirubin level at which exchange transfusion should be performed in neonatal jaundice of unknown aetiology is still unsettled. Most authors who recommend this procedure believe that it should be performed if the serum bilirubin level reaches or exceeds [illegible]0 mg [illegible]. Some estimate that the critical bilirubin level is [illegible]5 mg [illegible], others that it is 15 mg [illegible]. The lower level is especially applicable to premature children. The literature on this question has recently been reviewed (4).

Material and Method

During the six-month period, April 1 to October 1 1960 we systematically investigated serum bilirubin values in practically all jaundiced newborns. We examined only the jaundiced children since it is unlikely that newborn infants without jaundice will have a bilirubin value of 10 mg [illegible] or more and it was this group in whom we were interested. Daily serum bilirubin determinations were performed on heel blood. The micromethod used (Jendrassik & Grof) gives the total bilirubin concentration. The analyses were done at the Central Laboratory of Rigshospitalet (Head: Poul Astrup, M.D.).

In addition serological examinations were carried out on all jaundiced children and their mothers at the Blood Bank of Rigshospitalet (Head: Erik Fremblen, M.D.).

(a) *The newborn child*: ABO and Rh (D) group, direct Coombs test and Mørck Andersen conglutination test of the infant's red cells were found to be incompa[illegible] [illegible]

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the serum of the mother with respect t the ABO system.

(b) *Compatibility test between the child' red cells and the mother' serum.* The test was per formed with saline suspension in a test tube at 37°C and followed by an indirect Coombs test

( ) *The mother* ABO and Rh (D) group. Furthermore, the serum was examined at 37 C for irregular antibodies using saline suspensions from a large panel f known red blood cells, supplemented with an indirect Coombs test. If ABO group incompatibility was found, the mother serum was examined for specific haemolysins and immune anti bodies gainst the child' ABO antigen (neu tralization technique with indirect Coombs test)

In the six-month period of the study there were 1073 single births. As prophylactic measure all the children were given 2.5 mg Menadione intramuscularly immediately after birth. In order to calculat the incidence of jaundice, all stillbirths and neonatal deaths were excluded from the total, because all jaundiced children survived the first week of life. This gives us a corrected figure of 1000 newborns.

Of these 1000 newborns, 343 developed jaundice within the first week of life

- 36, i.e. 3.6%, due to haemolytic disease (morbus haemolyticus x rhesus incompatibilitat);
- 22, i.e. 2.2%, due to haemolytic disease (morbus haemolyticus ex ABO in compatibilitate)
- 305, i.e. 30%, due t jaundice of unknown aetiology (icterus ex causa ignota).

Results

Ninety five of the 305 infants with jaundice of unknown aetiology had serum bilirubin value > 15 mg %, and 65 chil dren had a serum bilirubin level of > 20 mg %. all had values of 10 mg % or more

The percentage of primiparae is the same in the group of infants with jaundice of unknown aetiology as it is in the group without jaundice

The frequency of prematurity (≤ 2500 g) for the 305 children with jaundice of un known aetiology was *4.2%, while the frequency for the remaining 695 was 6.5 % (When the 58 who were icteric due to Rhesus or ABO incompatibility are subtracted from the 695 th prematurity frequency is 6.4%.) Frequency of prematurity in the 65 infants with a serum bilirubin value > 20 mg % was 20%. Ex change transfusion was carried out in 36 of the 65 children, i.e. 55%, for these the prematurity frequency was 19%.

Thus, in the period under investigation 3 6% of the 1000 newborns had exchange transfusions because of jaundice of un known aetiology The ratio was 5.9% of the premature and 3.3% of the full-term infants. All who were not given exchange transfusion had a serum bilirubin concentration less than 25 mg %.

Despite the fact that this case material of our department in many respects is a selected one (e.g the high number of premature births) the percentage of chil dren with jaundice of unknown aetiology in both the full-term and premature groups is, by our present knowledge, representative of a non-selected group The very high percentage of exchange transfusions performed in this series leads one to question whether so many are necessary

It should be noted that the department is a centre for Rhesus immunized mothers, and of the 1000 children during the six month period plus 1970 corresponding infants from 1950 3 6% had haemolytic disease due to Rhesus incompatibility Eighty percent of these children had ex change transfusions, i.e. 2.9% of the

TABLE 1 *Schematic presentation of the calculations (To be read from th left to th right)*

	Number	
Out of	1,000,000	children aged more than one yea
there are	0.2 % = 000	with cerebral palsy
out of these *000	17.0 % = 340	have athetoid
out of these 340	70.0 *= 238	had severe jaundice in the first week of life
out of these 238	46.0 *= 109	had jaundice due to Rhesus-incompatibility
out of these 238	*0.0 % = 48	had jaundice due to ABO-incompatibility
out of these 238	34.0 = 81	had jaundice of unknown aetiology
out of these 81	33.3 = *7	were mature (that is 0.00*3 * of 95,000)
out of these 81	66.7 ,, = 54	were premature (that is 0.1*00 of 45,000)

20*0 children. Even if one takes into ac count the fact that the department is a Rhesus centre it will be seen that, in the six month study period, more infants were given exchange transfusions because of jaundice of unknown aetiology than because of Rhesus incompatibility (3 6 % against 2.0)

Calculati ns

In order to answer the question of how necessary it is to perform exchange transfusions in so many children with jaundice of unknown aetiology the following cal culations have been made for 1 000 000 non-selected and non-exchanged live-born infants on the ba is of the following in formation.

According to Hansen s follow-up study (3) the frequency f cerebral palsy in this country is about 0. ,, In Plum investigation (8) athetos is found in 17* of the children with cerebral palsy Moreover in his retrospect e study Plum showed that j st less than 0 of the hildren with athet osi had severe ja ndice during the first weeks f lif In 46 this was due to Rhesus incompat bility in *0 to ABO incompati bilit while in 34 there was no serological explanation. The incidence of prematurity (<2500 g) was 10%, 55 ,, and 64 ,, respectively

The incidence f premature births in 19.4 in this country was 5. ,, (5). Verrecuard' study (7) showed that between *0 and 3 of the premature births (by his definition <2500 g) die in the first year of life This means that at the age of one year the fre quency of premature births is 4.5 ,,

Of 1,000 000 new born 943,000 are full term and 57 000 premature (5.*), but of 1 000 000 children aged one year 9.. 000 will have been full term and 45 000 premature (4.5)

Do these figures agree with th ex change transfusions actually performed

Rh s s group

First the question mu t be ans ered f r the Rhesus group It is well known that maternal Rhesu antibodies are f und in 0.5 of all pregnancies In a study of Rhesus immunised mothers fr m th department it wa found that 8 had macerated or hydroptic babies (6) In other word among 1 000 000 live birth there will be 4000 infant f Rhesu immunised mothers What will happen t

these children if nothing is done for them? Vaughan, Allen & Diamond, (9) state that 34 % will die and 5 % of the survivors will have kernicterus Of the above-mentioned 4600 children, 1565 will die 151 will survive but will have kernicterus, and 2885 will be normal.

Since kernicterus manifests itself most frequently as athetosis, there is good correlation between the number of children with kernicterus, which we find within the untreated Rhesus group and the number of children with athetosis we estimate on the basis of Plum s retrospective study (151 and 100 respectively)

How many of these 4600 children would have been exchange transfused in our department? According to the above mentioned Rhesus material (6) this number would be 63 0 % since 12.9 % would be Rhesus negative 4.5 % Rhesus positive but not sensitized, and 19.6 % Rhesus positive and sensitized but exchange transfusion would be considered unnecessary In the same study we found that three of the 249 exchange transfused children died for unknown reasons during or immediately after an uncomplicated exchange transfusion, in spite of the fact that these children were no more sick than the remaining 46 We therefore must reckon with a mortality rate of 1 % due to the exchange transfusion itself. In other words 2898 infants would have been exchanged in order to avoid kernicterus in 151 and death in 1564 but the exchange transfusion itself might have caused the death of 29 of these Although we exchange transfuse slightly more than necessary there is no doubt that our indications for exchange transfusion are appropriate and sound.

Children with jaundice of unknown aetiology

It can be seen from the calulations in Table I that athetosis in children suffering from jaundice of unknown aetiology in the first weeks of life is 43 times more frequent in premature babies than in full term babies (0 1. % vs. 0.0023) Therefore the question of the necessity of exchange transfusion has to be investigated separately for the full term babies (>2500 g) and the premature babies (≤2500 g)

(a) *Full-term babies*

In the period under investigation 881 of the 1000 children were full-term. Jaundice of unknown aetiology was found in 231 or 26.0 %. Serum bilirubin values of ≥ 20 mg % were found in 52 (5 9 %) of the 881 children, and °9 of the 5_ children (56 %) were exchange transfused, i.e. 3.3 % of the 881

Applying these figures to the hypothetical 943 000 newborn full-term babies will mean that 247 066 will have a serum bilirubin of ≥10 mg % during the first

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Similar unexplainable deaths among Rh-sensitized children who had exchange transfusions have been reported from the Sloane Hospital. From 1947 through 1955, of 236 exchange transfusions, there were 4 unexpected deaths, total of 1 7 per cent From 1956 through 1958 among 126 such procedures there were 4 unexpected fatalities, total of 3.2 per cent Because of these unexplainable deaths it is concluded that one always also should consider the risk of the exchange transfusion itself. "Comparison of the risk involved, however is particularly important in infants with hyperbilirubinemia not primarily the result of hemolytic disease such as hyperbilirubinemia in prematures, for which the role of exchange transfusions has been less well evaluated than it has in cases of erythroblastosis fetalis. (Wolff, James A.: Discussion: *Bull Sloane Hosp Women* 4 93–97 1959)

week of life .. 63" a serum bilirubin of ≥20 mg ..., and 31 110 will be exchange transfused for fear that they will later develop athetosis

According to the calculations (Table 1) only 0 00.8 .. will later develop athetosis. To avoid this in "0 cases we would exchange transfuse 31 110 and if we had adhered strictly to the value of ≥20 mg ... the number would have risen to 55 03" Since the department had not previously carried out exchange transfusion in children with jaundice of unknown aetiology and since we know that these children did not die the enormous discrepancy shows that our indications for exchange transfusions are unsound.

It may of course be said that it is better to exchange transfuse too many rather than too few but here one must take into account the mortality rate in exchange transfusion, which for the full term babies is calculated to be 1 This would mean that if we decided to exchange transfuse all full term babies with serum bilirubin levels of "0 mg% of unknown aetiology we would have to exchange transfuse *140 children in order to prevent one from developing athetosis and would run the risk of losing .1 by the procedure

(b) *Premature babies*

Of the 1000 children in the period under investigation 110 were premature Jaundice of unknown aetiology was found in "4 (0..) and serum bilirubin values of ≥ 20 mg .. in 13 i.e. 11 of the 119 children, and " of the 13 children (.4) were transfused i.e. 5.0 .. of the 119

Applying these figures to 5" 000 newborn prematures would mean that 35,340 would have a serum bilirubin of ≥10 mg .. in the first week of life 0270 a serum bilirubin of ≥20 mg %, and 3363 would be exchange transfused to avoid the possible development of athetosis.

According to the calculations (Table 1), 0 12 .. should later develop athetosis i.e. 69 of the 57 000 premature infants Thus, to avoid athetosis in 69 3363 would be exchange transfused or 0270 if the limit of ≥ 20 mg .. is strictly adhered to. We would thus have to exchange transfuse 9 children in order to save one From the Rhesus material in this department (6) we know that exchange transfusion carries a mortality rate of 4 % in premature babies In other words, if we decide to exchange transfuse all prematures with a serum bilirubin of ≥20 mg .. of unknown aetiology we run the risk of having 4 infants die for each one we prevent from developing athetosis by performing an exchange transfusion.

The conditions are however difficult to evaluate because it is quite possible that there are more children with athetosis among those dying in the first year of life than among the survivors But how many? From the Rhesus group we know that if we choose not to exchange transfuse it means that a number corresponding to a good 10 times the number with kernicterus will die If we use this number we would save 69 + 704 = 772 by exchange transfusion 6770 i.e., save 10 for each 90 transfused, but we would run the risk of 4 dying because of this procedure

This figure corresponds exactly ... has found by Dyggve (1) ... prophylactic treatment of premature children ... 1 mg K vitamin given intramuscularly immediately after birth.

ABO group

Finally the question has been investigated with reference to the ABO group In our material of 1000 children there were 22 jaundiced children in whom A or B antibody could be demonstrated (*... %) (This figure agrees with an earlier consecutive study of 3,600 neonates from this department (*) in which 2.0 % with A or B antibody were found, 84 % and 16 %, respectively As far as the demonstration of A or B antibodies is concerned, both study groups were non-selected.) Of the 22 children 9 were exchanged, i.e. 0.9 % of the 1000 children. Here there is also a great discrepancy between the number of exchange transfusions performed and the figures based on Plum s study The same considerations mentioned in the section on infants with jaundice of unknown aetiology apply here.

Discussion

The criteria for exchange transfusion of Rhesus-sensitised babies are well-founded.

Even if one assumes it to be a fact that full-term infants with jaundice of unknown aetiology may later develop athetosis, we do not know today which children should be exchange transfused. It seems justifiable not to exchange transfuse the children simply because the serum bilirubin values reach 20–25 mg %. If it rises to over 30, 35 or 40 mg % it is, with our present knowledge questionable whether one dares to omit an exchange transfusion Both the non-exchange transfused infants and those transfused should be followed up.

Our criteria for performing transfusions on premature infants with jaundice of unknown aetiology and children with jaundice due to ABO incompatibility are unsatisfactory

Cases of kernicterus in premature infants with serum bilirubin values as low as 12.6 mg % have been reported (4) Therefore it does not appear that the serum bilirubin concentration alone is the decisive factor Other factors must also be involved. For example it is not yet known whether it is a rapid rise in serum bilirubin value (e.g more than 20 mg % within the first 48 hours) the duration of a severe jaundice or a prolonged jaundice alone, that is decisive for the brain damage. Perhaps the amount of indirect bilirubin plays a part. It is possible that the percentage of haemoglobin, reticulocyte count and amount of foetal haemoglobin in the newborn are of importance, and possibly the amount of blood transferred from the placenta to the infant before the cord is tied also plays a part. It is difficult to say at the moment, what one should do, but the premature infant runs a greater risk of developing athetosis than does the full term infant. A systematic investigation, including follow up, must be carried out in order to solve the problem.

Summary

In a study of 1000 newborns jaundice of unknown aetiology was a common finding during the first week of life. In these children the bilirubin values were as follows:

$>$10 mg % in { 26 % of full-term; 62 % of premature }

$>$*0 mg % in { 6 % of full-term; 11 % of premature }

It is known from the literature that there is a causal relationship between

severe jaundice occurring in the first week of life and the subsequent development of athetosis. By carrying out exchange transfusion on children with a serum bilirubin value of ≥ 20 mg % and comparing the number with the frequency of athetosis reported in the literature it is shown that the following will be exchange transfused

(a) 2140 full term babies in order to prevent subsequent athetosis in one but one runs the risk of 21 dying as a result of the procedure itself

(b) 0– premature infants to prevent subsequent athetosis in one but one runs the risk of 4 dying a a result of the procedure

The limit of 20 mg % is thus not appropriate Through systematic investigations must be found better criteria for exchange transfusing newborn with jaundice of unknown aetiology

As opposed to this the criteria for exchange transfusing Rhesus sensitised children are satisfactory Admittedly it is unnecessary in the case of 41 out of every 100 children but on the other hand, 6 will be saved from death or kernicterus with the risk of death due to the procedure in only one case

Addendum

It has been tated that j undice f unknown aetiology is rare finding in premature infants of t emic mothers (R. Sacrez, J M Lévy E. Scheppler & M. Klein: Relations entre l'ictère physiologique d prématuré et la toxémie tardive de la grossesse Sem Hôp (Paris) (Ann Péd) 36 1 19 1960). Our investigation can neither confirm nor weaken this observ tion, since only two mothers w th toxemia gave birth to premature babies who survived the first week. One infant (1450 g) had it maximum serum bilirubin value (10 4 mg%) on the 4th day and the other (100 g) was not jaundiced. As fa the mature babies are concerned our investigation shows that jaundice of unknown aetiology occurs just as frequently in infants of toxemic mothers as in other mature babies. There were 4 mothers th toxemia (both mild and severe) and 11 of these infant had j undice and serum bilirubin al of > 10 mg% (three were ex hange transfused) Thus the frequency of j undice of unknown aetiology was 3 4 in infant of toxem moth ers and 26.3 in infant of mothers th ut toxemia.

References

1 Dragovy H Bilirubin studies in premature infant who recei ed menadione deriva t es or itamin h t birth. Acta Paediat 49 230 1960

2 Dragovy H and Mex Andersen O On the incidence of haemolytic disease of the newborn due t ABO incompatibility Acta Paediat 49 19 1960.

3. H s Er Cerebral Palsy in Denmark. Thesis, Munksgaard, Copenhagen, 1960

4 Kalla den, A., Mi ller Larsen and U., and Srölis B Indications for exchange transfusion in newborn infant with hyperbilirubinaemia not due to Rh immunization. Acta Paediat 49 377 1960

5 Kare T Perinatal dødelighed og dens geografiske relationer Studier over den perinatale dødelighed. Ugeskr Læger 120 593 1958

6. Lindow u., F Snare re B and Trolle, D P per read t the Board (Gynaecology Finland, 1960

7 Nørgaard and B Anæmie u f fødsel. Thesis. Ernst Hansen, Copenhagen, 1953.

8 Perm Pa Etiology of congenital athetosis Paediat Fenn 3 549 1957

9. Yates w \ C., Allen F H and Diamond L K: Erythroblastosis fetalis I. Problems in the interpretation of stati ing mortality in erythroblastosis fet a Pediatrics 6 173 1950

10. Erythroblastosis fetalis IV Further o serva on on kernicterus Pediatrics 6 708 1950

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CASE REPORT

Successful Resuscitation of Two Immature Infants with Cardiac Arrest During Exchange Transfusion

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Exchange transfusion, still the only adequate treatment for hyperbilirubinemia of infancy carries a considerable risk to the patient. The overall frequency of complications has been estimated to be as high as 40 % [17] and it has been stated [1] that in more than 3 % of the transfused cases the complication has been severe enough to indicate discontinuation of the transfusion. Diamond emphasizing the importance of optimal technical conditions states that it should be possible to keep the mortality of exchange transfusions to less than 1 % [5].

Fatal accidents in connection with exchange transfusions are in most cases due to circulatory collapse caused by cardiac arrest and both ventricular fibrillation and ventricular asystole have been described (13 16). The pathophysiology of these fatal accidents is not well understood, although it has been thoroughly studied [–, 4 9 10 14], and no single parameter has thus far been considered responsible.

The present communication describes two instances of cardiac arrest during exchange transfusion, both successfully treated with cardiac massage after efforts with the more conventional methods of resuscitation had failed. It is the authors aim to show that a cardiac arrest in infants does not necessarily mean a fatal outcome and that adequate treatment of these accidents during exchange transfusions may even further decrease the mortality still attendant to the procedure.

Case Reports

The first infant was the fourth offspring of a healthy mother and belonged to blood group A Rh neg. The mother was O Rh pos. Her previous three children were alive and well. The pregnancy which had been uneventful, terminated at 34 weeks with the birth of an infant weighing 2160 g. The delivery and the immediate neonatal period were normal. On the first day a slight icterus was noted, which had increased on the second day of life. The indirect serum bilirubin was 18 mg/100 ml. Because of the early hyperbilirubinemia, the immaturity of the baby and the presence of high titers of anti-A antibodies in the mother blood an exchange transfusion was performed. Four hundred ml of type 0 red cells in A Rh neg plasma were given through the umbilical vein in lots of 20 ml. During the procedure the child was in very good condition. A second exchange was performed on the third day of life because of an indirect bilirubin of 20 mg/100 ml.

This transfusion was also completed without any difficulties.

On the fourth day of life a third exchange transfusion was performed because the indirect bilirubin level was still at 20 mg/100 ml. When 300 ml had been transfused the infant suddenly without any previous warning symptoms or signs, gasped or 3 times, and became pale-cyanotic. The transfusion was immediately discontinued and auscultation of the heart revealed no heart sounds. Efforts to initiate heart action were immediately started: the arms and legs were elevated the head lowered, the thorax was violently compressed antero-posteriorly over the heart and the precordial area was slapped repeatedly while oxygen was administered by mask with positive pressure. These procedures were unsuccessful and after approximately 45 seconds a thoracotomy was performed over the heart in the 5th intercostal space. There was virtually no bleeding. The heart was dilated and without apparent contractions.

Without opening the pericardium, cardiac massage was started with the flaccid heart rhythmically pressed between the operator's forefinger and the anterior thoracic wall. After five compressions the tonus of the heart improved and within ten cycles there were spontaneous contractions of the ventricles. Within a few minutes the heart rate was 150 per minute.

The effect of the return of cardiac action was startling: with the first few strokes the color of the skin became pink and the infant started to cry loudly. Endotracheal intubation was not performed for approximately 5 minutes. Despite the fact that the infant was using only one lung until intubation, it nevertheless oxygenated fairly well and was capable of relatively vigorous, intermittent crying.

After cardiac action had resumed the bleeding was controlled, oxytetracycline was instilled into the pleural cavity, the collapsed lung was inflated and the wound was closed in layers. During this procedure a compensatory transfusion of 50 ml blood was given. Continuous suction was applied to the left pleural space; the child was extubated and started on antibiotics.

The recovery of the infant was entirely uneventful. The pleural suction was discontinued after 24 hours. Electrocardiograms revealed normal standard and precordial leads. Despite repeated and thorough neurological examinations no signs of CNS damage were noted. The serum bilirubin fell steadily. The general appearence and attitude of the child was not different from that of a normal child; she gained weight steadily and was discharged from the hospital on the 20th day of life weighing 2210 g. Follow-up examination at the age of 3 months was entirely normal.

The second infant, an immature female twin with a birthweight of 1700 g and belonging to blood group A Rh pos, was delivered subsequent to extraction of its dead twin brother. The delivery terminated an uneventful 33 week pregnancy. The immediate neonatal period was uneventful but on the third day of life she developed icterus with an indirect bilirubin of 10 mg/100 ml, which increased to 1.5 mg/100 ml on the fourth day of life. The child was then admitted to the children's clinic for exchange transfusion. The mothers blood, type 0 Rh pos, contained anti-A antibodies and an exchange transfusion was performed with 0 Rh pos rbc in A Rh pos plasma. The infant was in good condition when the transfusion was started, cried vigorously and did not show any signs of circulatory or respiratory disturbances. The transfusion was performed through the umbilical vein and the administered blood was thermostatically heated to 36°.

When the transfusion was almost completed and 220 ml blood had been exchanged, the child suddenly became pale-cyanotic and stopped breathing after 2–3 gasping inspirations. The head was immediately lowered, oxygen was administered by mask with positive intermittent pressure without apparent effect upon the color of the skin or upon the general condition of the child. Auscultation over the heart revealed no heart sounds and efforts to reinstitute regular

heart action were immediately started. The legs and arms of the infant were raised to provide increased venous return to the right heart, vigorous compressions of the thorax over the heart were performed rhythmically and the precordium was repeatedly beaten while oxygen was given with intermittent positive pressure. When after two minutes these procedures had had no effect upon the child who remained lifeless and pale-cyanotic, a thoracotomy was performed.

The incision was made along the 5th inter space in the precordium and when the thoracic cavity was incised and the lung had collapsed, the heart was found to be completely dilated, bluish grey in color and without apparent contractions. When the heart was palpated it gave the impression f being completely atonic without vibrations or contractions.

Massage was started with the heart between the operator forefinger and the anterior thoracic wall. After 10 compressions the tone began to return and these first ten compressions had dramatic effect upon the general status of the child. The color of the skin improved and the child began to take deep v luntary inspirations. Eye movements returned. There was, however still no spontaneous heart action seen or f it. At this time endotracheal intubation was at tempted unsuccessfully and intermittent positive pressure with oxygen by mask was continued. After five minutes of massage t a rate f 80/min the heart still did not ha e any contractions of its own. The infant was react ing reasonably normally with fforts to cry and making regular breathing movements with her right lung. Three ml of Ca gluconat was now injected into the right ventricle and massage was continued. When t seven minutes still no spontaneous heart action was apparent, 0.2 mg adrenalin was injected intracardially After total of 10 minutes of massage the first heart action was felt and ventricular contractions t a rate of 20/min started.

Manual massage was continued until the spontaneous heart rhythm at 14 minutes had reached approximately *00/min. The child now cried vigorously and the cyanosis completely disappeared. Slight bleeding started from the margins of the wound but could easily be controlled. The pediatric surgeon completed the wound closure and established pleural drainage. A transfusion f 20 ml was given with an apparently beneficial effect on the infant, whose skin became more pink. Antibiotics were administered, X-ray of the chest revealed good expansion of the left lung

The recovery of the patient was remarkably uneventful and the day after the episode the infant was able to drink from the bottle and appeared perfectly well. No signs f CNS damage were observed during the hospital stay that lasted for another two weeks, nor were there any at a follow up examination one month later Electrocardiographic tracings revealed no signs of myocardial damage.

Discussion

The authors do not wish to speculate as to the cause of cardiac arrest during ex change transfusions as these problems will be dealt with in a forthcoming communication. The present discussion will be limited to some problems associated with the treatment of the cardiac arrest in infants.

The finding in both the reported cases of a hypotonic dilated heart denotes the presence of ventricular asystole a situation that in contrast to ventricular fibrillation should be treated only by mechanical stimulation [8]. Although thoracotomy with intermittent manual compression of the heart must be considered to be the most effective mechanical stimulus and also the most effective circulatory adjuvant in cardiac arrest, the treatment of the asystolic heart has recently been the subject of several communications, report

ing good results from various procedures not involving thoracotomy. These include methods such as compressing the heart intermittently between the anterior thoracic wall and the spinal column [6, 15], violent compression of the upper part of the abdomen against the diaphragm [11], forceful beating on the precordium [3] and increasing the venous return to the asytolic heart by raising arms and legs of the patient [18]. The mechanical stimulation that a needle introduced into the myocardium through the thoracic wall creates has been suggested as an activator of spontaneous heart action [7], while injection of drugs such as calcium and adrenalin are supposed to be effective only when the injection is accompanied by effective cardiac massage.

All these methods have in common the fact that they are relatively easy to perform rapidly and do not require operative facilities. The great danger inherent in their application is, however, that valuable and perhaps vital time is lost if they are continued unsuccessfully. This may completely invalidate the beneficial effect of a successful intrathoracic treatment performed later by causing permanent CNS damage to the patient. Only if an adequate circulation can be maintained by extrathoracic methods, as has been reported for the method using thoracic compression [6, 15] is one justified in continuing for some length before thoracotomy is performed. The other methods should be tried quickly only once and if unsuccessful be followed by thoracotomy, manual cardiac massage and the administration of adrenalin and calcium [12].

The amazingly good effect upon the circulation that could be instituted and maintained by the one-finger massage on the dilated and hypotonic myocardium indicates that the small dimensions of the immature infant do not constitute a contraindication to thoracotomy and this opinion is further supported by the rapidity and ease with which these small infants recovered from the operation. Of particular interest in this connection is the fact, that spontaneous cardiac action could be reinstituted without first establishing an effective ventilation and oxygenation by means of endotracheal intubation together with the finding that both these infants once they had regained their cerebral function could maintain a seemingly sufficient ventilation with only one functioning lung. Both these findings are somewhat unexpected and would indicate that immediate endotracheal intubation is not imperative. This would mean that the immediate problem is one of the return of cardiac action and all efforts should therefore be directed towards that end alone.

It has been the aim of this report to emphasize that thoracotomy may be a lifesaving procedure even in early infancy and should be considered as such and be performed without delay by the physician who recognizes the situation, when "external" methods have failed. Whatever the cause of the cardiac arrest may be it seems reasonable to suspect that cardiac standstill even if not pre-signaled by alarming changes in the general status of the infant, is preceeded by electrocardiographic changes. For this reason we would strongly recommend that the ECG be continuously monitored routinely throughout an exchange transfusion.

Summary

Two instances of asystolic cardiac arrest in immature infants during exchange transfusions are reported. In both cases the collapse came as an unexpected complication at the end of the transfusion. When extrathoracic manipulations to restore spontaneous heart action had been unsuccessfully performed for one and two minutes respectively thoracotomy was performed and cardiac massage instituted. Spontaneous heart action started in one case shortly after manual compressions had been instituted and in the other case after 10 minutes of massage and the administration of calcium and adrenalin. The recovery of the infants was completely uneventful and no signs of CNS damage could be detected at the age of two and three months respectively. Methods to institute cardiac action are discussed and it is stressed that thoracotomy and cardiac massage are comparatively easy procedures not contraindicated by the small size of these infants. The use of continuous ECG control during exchange transfusion is recommended.

References

1. Boggs, Th. R. and Westphal, M. C.: Mortality of exchange transfusion. *Pediatrics, 26* 745, 1960
2. Boland, R. P., Trapeman, H. S. and Philipsborn, H. F. Jr.: Electrolyte considerations in exchange transfusions for erythroblastosis fetalis. *J Pediat, 49* 401 1956.
3. Brandenburg, J. T.: Successful treatment by chest blow of cardiac arrest during myocardial infarction. *JAMA, 170* 1907 1959
4. Campbell, W. A. B.: Potassium levels in exchange transfusion. *Arch Dis Child, 30* 513, 1955.
5. Diamond, L.: Letter to the Editor *Amer J Dis Child, 100* 940, 1960
6. Eastwood, X. B.: External cardiac massage. Letter to the Editor *Lancet, 1* 544, 1961
7. Editorial review. Treatment of cardiac arrest. *Brit Med J 1* 409, 1960.
8. Editorial review. Cardiac arrest and resuscitation. *Lancet 2* 1333, 1960.
9. Farquhar, J. W. and Smith, H.: Clinical and biochemical changes during exchange transfusion. *Arch Dis Child, 33* 142, 1958.
10. Graham, B. D. and Hey, R. M.: Acid base homeostasis during exchange transfusion of newborn infants with preserved blood. *Pediatrics, 15* 241 1955.
11. Healy, J. B.: External cardiac massage. Letter to the Editor *Lancet, 1* 608, 1961
12. Hosler, R. M.: Manual on Cardiac Resuscitation. Ed. — Ch. C Thomas Publ., Springfield, Ill. 1959
13. LeVeen, H. H., Pasternack, H. S., Lustrin, I., Shapiro, R. B. and Yecker, E.: Hemorrhage and transfusion as the major cause of cardiac arrest. *JAMA, 173* 770, 1960.
14. Miller, G., McCoord, A. B., Joos, H. A. and Clausen, S. W.: Studies of serum electrolyte changes during exchange transfusions. *Pediatrics 13* 412, 1954.
15. Molloy, P. J.: External cardiac massage. Letter to the Editor *Lancet, 1* 49, 1961.
16. Taylor, W. C., Grisdale, Ch. B. and Stewart, A.: Unexplained death from exchange transfusion. *J Pediat 52* 694, 1958.
17. Walker, W. and Neligan, G.: Exchange transfusions in haemorrhagic disease of the newborn. *Brit Med J 1* 681, 1955.
18. Woodward, W. W.: Cardiac arrest treated by elevation of the limbs for fifteen seconds. *Lancet 2* 1120, 1960.

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CASE REPORT

Hamman–Rich Syndrome

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In 1935 Hamman & Rich (16) published the first cases of idiopathic, diffuse interstitial fibrosis of the lungs but it was not until after 1944 (17) when they described the syndrome again, that an interest in this disease was aroused. Since then about 100 cases have been reported. In 1957 Rubin & Lubliner (34) collected a total of 64 cases, partly from the literature and partly their own. A few of these however also suffered from other diseases which might have contributed to the lung disease. A more critical report was published in 1959 by Donohue *et al* (8) who collected 87 cases from the literature and added 10 cases of their own. Of these 97 cases only 12 were children. The syndrome thus seems to be very rare in childhood or at least rarely recognized as such.

Case Report

J 811/59 Girl, born in 1949 as the second child. The sister is well. No family history of heart or lung diseases. Development was normal. In December 1955 she was operated on for perforated appendicitis. Before operation tachycardia was observed, but no other symptoms of heart or lung diseases. Since the operation she has had increasing cyanosis, loss of appetite and a tendency to fainting during effort but no edema, and no cough. In August 1957 she was admitted to the Pediatric Department at Sundby Hospital.

Physical Findings

Cyanosis of the lips and dyspnea, slightly enlarged liver but no edema and no drumstick fingers. BP100/50. Auscultation of the lungs was normal. Auscultation of the heart revealed a gallop rhythm in the 4th left interspace and a faint systolic murmur over the precordium. When readmitted to the hospital in 1958 and 1959 she complained of a tendency to sweating, a feeling of cold feet, headaches and a short dry cough, particularly at night. The condition gradually progressed. From November 1958 edema and ascites were also observed but still there were no signs of drumstick fingers or watch-glass nails. Examination of the heart demonstrated enlargement 2 cm lateral to the mid-clavicular line. A loud, harsh systolic murmur was heard throughout the precordium. Auscultation of the lungs was normal. Death occurred in November 1959.

Laboratory Examinations 1957

X ray examination: lungs normal, heart moderately enlarged. Electrocardiogram: right axis deviation. Electrophoresis of serum protein was normal. Blood urea 28 mg%. Arterial oxygen saturation 96%. Erytrocytes 6.09, 6.23, 5.9 million. Leukocytes 16,300, 12 000 and 17 700 mm³. Differential



Fig. 1. Microscopy from the lung. Note the diffuse pronounced fibrosis of the alveolar septa. (Gieson–Hansen; 73).

count normal. Urine revealed varying proteinuria (maximally 0.2 %). Heart catheterization (performed by Ib Boesen, M.D., at Dronning Louises Bornehospital) showed pulmonary hypertension and an increased diastolic pressure in the right auricle and right ventricle, plus low oxygen saturation in the venous blood.

September 1959

X-ray examination of the thorax (Fig. 2): lungs normal, heart enlarged with a prominence of the pulmonary arch, the right auricle and the right ventricle. Blood pressure 85 systolic. ECG showed right axis deviation, Q 4 mm, T_{1-2} flat, T isoelectric, T_{V2-4} negative, qRs pattern in V_{2-4}, RS pattern in V_6. Rbc 6.2 million, leukocytes 8040; differential count, neutrophilia. Urine normal, but two days before death proteinuria again appeared. Treatment: digitalis, mercurial diuretics, chlorothiazide and penicillin, without any certain effect. Steroids were not given.

Autopsy

This was performed by Hemming Poulsen, M.D. Fifteen hundred ml of serous fluid were found in each pleural sac. The surfaces of the serous membranes were smooth and glossy without fibrinous coating. Lungs medium-sized, with slight emphysema in both upper lobes, and with slight atelectasis in the inferior lobes. Specimens of the tissue from all pulmonary lobes floated on water. Incision through each pulmonary lobe revealed no macroscopic focal changes, in particular no pneumonic processes. The consistency was not increased and there was no edema. Trachea and bronchi were normal. The pulmonary artery showed slight atheromatosis at the bifurcation, but no changes in the branches. The heart measured 8 9 cm and weighed 670 g. The endocardium was normal. The tricuspid valve was dilated and measured 11 cm cut open. Both the right ventricle and the right auricle showed a very high degree of dilatation. The wall of the right ventricle was also markedly hyper



Fig. 2. X-ray of the hest 2 months before death. Not the hrease of pulmonary fibrosis. H = right side

trophic and measured 7 mm upwards and 11 mm downwards in thickness. The papil lary muscles were much thickened. The pulmonary ostium was natural The left heart was normal. In the other parenchymatous organs there was marked congestion. There was moderate edema of the lower extremities. There was 1000 ml of clear light ascitic fluid and 150 ml of clear fluid in the pericardium.

Microscopy f the lung tissue (Fig 1) All the lung lobes showed essentially the same histological picture although the degree of the changes aried considerably The hanges were most pronounced in the lower lobes, and in each of the lung lobes subpleurally Connect ve tissue increase was seen in the alveolar walls, at some places with many collagenous fibrils, at other points with many fibroblasts. Dilated, blood filled capillaries were ften observed, wherea there were only a few small, and dispersed infiltrations of lymphocytes. Granulocytes or plasma cells were not found. The alveolar epithelium was flat to cuboid. There was no edema, but a number of macrophages containing blood pigments were found in many alveoli. Hyaline membranes were not found. The bronchi showed a slight increase of the connective tissue of the adventitia, but no inflammation. In the branches of the pulmonary artery proliferation of the connective tissue occurred in the adventitia as well as in the muscularis mucosa. The intima was within normal limits. There were no thromboses nor muscular hyperplasia. Elastic tissue staining (11) showed no abnormalities. Microscopy of the heart revealed rather pronounced fibrosis of the myocardium. No inflammatory cells were present. Microscopy of cerebrum demonstrated edema and acute and chronic anoxemic changes.

Discussion

Clinically Hamman–Rich syndrome is characterized by cough, fever loss of weight, tendency to perspiration, cyanosis and dyspnea (34) all of which are non-specific symptoms of heart and lung disease X ray examination of the thorax

TABLE 1 *Previously described cases of Hamman–Rich syndrome in children verified by lung microscopy*

		Sex	Age at death
Lunseth *et al.*	(1955)	F	11 weeks
Bradley	(1956)	F	9 years
Grant *et al.*	(1956)	F	13 years
Donohue	(1956)	F	1½ years
		F	7 weeks
Aronson	(1956)	M	9 years
Obrecaj *et al.*	(1956)	F	?
		F	3 years
		F	8 years
Baar *et al.*	(1957)	M	2 years
Fekorrman *et al.*	(1957)	M	?
		F	7 months
Donohue *et al.*	(1959)	F	6 months
		F	1 year
		F	8 months
Mann	(1960)	F	8 months
Wilson *et al.*	(1960)	M	3 months
		F	1 month
		+ 2 cases, not described	

shows varying, non-specific lung infiltrations (6 10 30 34) or may even be normal (19 31) Cor pulmonale is seen in about 50% of the cases (30) The laboratory tests do not give any definite support to the diagnosis (20 34) Cardiac catheterization shows increased pressure in the right ventricle and pulmonary artery and reduced arterial oxygen saturation (36). In our case the pressure was increased but the arterial oxygen saturation was normal and there was a large arterio-venous oxygen difference as is seen in primary pulmonary arterial hypertension (9)

Only microscopic examination of the lung tissue can give the correct diagnosis (6 30 32) The microscopic picture is first and foremost characterized by a diffuse interstitial increase of connective tissue, metaplasia of the alveolar epithelium cells, thickening of the alveolar septa because of proliferation of fibroblasts dilatation and proliferation of capillaries and edema. Sometimes hyaline membranes are seen. Either no or only slight organization of alveolar exudate is seen. The vessels are often moderately hypertrophic because of hyperplasia of the intima or media (14 16 17 32)

As has been stated previously the etiology is unknown. Fibrosis of the lungs as such is a non-specific reaction (38) which may be caused by numerous agents (6), including infections inhalation, systemic diseases circulatory disturbances and irradiation. Some authors, for example, Baar *et al.* (2) mention collagenosis as an etiologic possibility but the microscopic picture of the lung tissue in collagen diseases seems, however to be slightly different from what is seen in the Hamman–Rich syndrome (10 23 27 33) Several authors, among others Pokorny *et al* (32), believe the cause to be due to insufficient resolution following one or more attacks of interstitial pneumonia because of a defect in fibrinolysis. Proof of this theory is still lacking Other authors mention the possibility of a hereditary disposition (4 38) or a hereditary congenital defect of lung tissue (8). Of the reported cases about 15% have had a familial occurrence. Congenital dysgenesis of the lung tissue may well explain the cases in infants.

In Table 1 are listed the previously reported cases in children in which microscopic examination of the lung tissue is reported. Donohue's two cases (7) and the case published by Lunseth *et al.* (22) were previously described as interstitial plasma cell pneumonia, but Donohue *et al.* (8) later revised these diagnoses when no pneumo-

cystis carinii were isolated and now consider them a form of pulmonary dysgenesis. Wilson *et al.* (39) state that the microscopic picture in their four cases in prematures is quit like the picture in Hamman-Rich syndrome but all the same they call them "a new form of respiratory disease" as n cases have been reported in the neonatal period. This point f view is hardly correct as there are descriptions of cases in children in all age groups including early infancy The existence in the neonatal period fits well with the theory advanced by Donohue *et al.* of congenital pulmonary dysgenesis (8)

In addition to the cases listed in the table there are other cases in the literature where the diagnosis is more uncertain or even doubtful. This is true about Březina s four cases (4) one of the cases described by Donohue *et al.* (8) and about Levinský s case (1), because microscopic examination of the lung tissue was not performed in these cases. The case reported by Mario *et al.* (6) differs both macroscopically and microscopically from the usual picture but may be considered as a variant of Hamman-Rich syndrome On the other hand, the diagnosis in Diamond s case (5) is hardly correct as has also been pointed out by Donohue *et al.* (8). In addition to fibrosis of the lungs Diamond found rather pronounced changes in the vessels, which may have been the primary cause of the lung disease.

Of the certain cases listed in Table 1 and our own case 15 are girls and only four are boys This does not correspond to the distribution found among adults where the number of cases among men and women is equal (8) The unequal distribu tion must, however be evaluated with some caution because of the few cases reported among children.

In some cases treatment with cortisone has shown to have a good effect most of ten it has only given subjective improvement (1° 13 18 ºO 37) but in a few cases an improvement in the X ray appearance of the lungs has been reported (29 31 35) On discontinuation of the treatment death has occurred a few days later in some cases, for which reason it must be recommended that treatment be continued without any regard to a lacking effect (ºO 29 35). In order to achieve an effect with cortisone it is important to start treatment as early as possible in the course of the disease, since steroids can only delay the prolifera tion of the fibroblasts, and are without effect once fibrous tissue is formed (20 29, 31)

Without cortisone the prognosis is bad, but the duration of the disease may vary from weeks to many years (8). It may be possible to improve the prognosis somewhat with cortisone treatment (31 37), but the observations to date are too few and of too short duration to permit a final evaluation.

Summary

A case of the Hamman-Rich syndrome in a 10 year old girl is reported. The duration of the disease was 5 years. Microscopy of the lung tissue showed an increase of connective tissue and dilated capillaries in the alveolar walls. Treatment with digitalis, penicillin and diuretics were without effect. A survey of previously reported cases of this syndrome in children is given

References

1. ABRAMSON, A.: Hamman–Rich syndrome. J Maine Med Ass 47: 105, 1956.
2. BAAR, H. S. and BRAID, F.: Diffuse progressive interstitial fibrosis of the lungs in childhood. Arch Dis Child 32: 199, 1957.
3. BRADLEY, C. A.: Diffuse interstitial fibrosis of the lungs in children. J Pediat 48: 442, 1956.
4. BRENNTA, Z.: Über Lungenfibrose im Kindesalter. Kinderaerztl Prax 27: 57, 1959.
5. DIAMOND, L.: The Hamman–Rich syndrome in childhood, report of a case with unilateral pulmonary arterial and venous stenosis and tricuspid occlusion. Pediatrics, 22: 279, 1958.
6. DONOGHUE, F. E. and WOOLNER, L. B.: Pulmonary fibrosis. Med Clin N Amer 1009, 1954.
7. DONOHUE, W. L.: Interstitial plasma cell pneumonia. Lab Invest 5: 97, 1956.
8. DONOHUE, W. L., LASKI, B., UCHIDA, I. and MUNN, J. D.: Familial fibrocystic pulmonary dysplasia and its relation to the Hamman–Rich syndrome. Pediatrics, 24: 786, 1959.
9. DRINSDALE, D. T., SCHULTZ, M. and MICHTOM, R. J.: Primary pulmonary hypertension. Amer J Med, 11: 686, 1951.
10. ELLMAN, P. and CUDKOWICZ, L.: Pulmonary manifestations in the diffuse collagen diseases. Thorax, 9: 46, 1954.
11. ENGELUND, V.: Fast two-step trichrome staining for paraffin and frozen sections combining demonstration of elastic fibres, fibrin and mucus with v. Gieson stain. Acta Path Microbiol Scand, 40: 105, 1957.
12. FEINERMAN, B. and HARRIS, L. E.: Unusual interstitial pneumonitis. Proc M[?] [?] Cl[?] 32: 637, 1957.
13. FRIEDMAN, A. and DEATON, W. T.: Diffuse interstitial fibrosis of the lungs (Hamman–Rich syndrome). Successful palliation of a case with steroid therapy. Dis Chest 34: 857, 1958.
14. GOLDEN, A. and BRONK, T. T.: Diffuse interstitial fibrosis of lungs. Arch Int Med, 92: 606, 1953.
15. GRANT, I. W. B., HILLIS, B. R. and DAVIDSON, J.: Diffuse interstitial fibrosis of the lungs (Hamman–Rich syndrome). Amer Rev Tuberc 74: 485, 1956.
16. HAMMAN, L. and RICH, A. R.: Fulminating diffuse interstitial fibrosis of the lungs. Trans Amer Cli Climat Ass, 51: 154, 1935.
17. HAMMAN, L. and RICH, A. R.: Acute diffuse interstitial fibrosis of the lungs. Bull Johns Hopkins Hosp, 74: 177, 1944.
18. HARRIS, M. S., PRETTS, F. S., GOLDMAN, A. and FRIEDMAN, N.: Cortisone therapy in Hamman–Rich syndrome. Amer Rev Tuberc 76: 122, 1957.
19. HEPPLESTON, A. G.: Chronic diffuse interstitial fibrosis of the lungs. Thorax 6: 426, 1951.
20. HOFF, H. R.: The Hamman–Rich syndrome. New Engl J Med 259: 81, 1958.
21. LEVINSKY, L.: Chronické difusní intersticiální fibrosa plic (idiopathická). Čas Lék Čes, 92: 978, 1953.
22. LUNSETH, J. H., KIRMSE, T. W., PREZYNA, A. P. and GERTH, R. E.: Interstitial plasma cell pneumonia. J Pediat, 46: 137, 1955.
23. LUTTON, M. J. and KUZMA, J. F.: Rheumatic fever pneumonitis: A clinical and pathologic study of 35 cases. Ann Int Med 44: 237, 1956.
24. MACMILLAN, J. M.: Familial pulmonary fibrosis. Dis Chest, 20: 426, 1951.
25. MANN, T. P.: Diffuse progressive interstitial fibrosis of lungs in infancy. Proc Roy Soc Med, 52: 638, 1959.
26. MARIE, J., HENNEQUET, A., HÉBERT, S. et BOCQUET, L.: Sur une variété particulière de fibrose pulmonaire subaiguë de l'enfant, vu aspect cirrhotique de la surface des poumons. Sem Hôp Paris, 33: 4018, 1957.
27. MCGEHEE HARVEY, A., SHULMAN, L. E., TUMULTY, P. A., CONLEY, C. L. and SCHOENRICH, E. H.: Systemic lupus erythematosus: Review of the literature and clinical analysis of 138 cases. Medicine (Balt), 33: 291, 1954.
28. OSSAKAJ, W. and MICHAL, L.: Postępujące śródmiąższowe zwłóknienie płuc wieku dziecięcego. Pediat Pol, 31: 509, 1956.
29. PEABODY, J. W., BUECHNER, H. A. and ANDERSON, A. E.: Hamman–Rich syndrome. Arch Int Med 92: 806, 1953.
30. PEABODY, J. W. and PEABODY, J. W. J.: Diffuse fibrosing interstitial pneumonitis (Hamman–Rich syndrome). Dis Chest 25: 709, 1954.
31. PINNEY, C. T. and HARRIS, H. W.: Hamman–Rich syndrome. Amer J Med 20: 308, 1956.
32. POHORNY, C. and HELLWIG, C. A.: Diffuse interstitial fibrosis of the lungs. Arch Path, 48: 352, 1955.
33. PRICE, T. M. L. and SKELTON, M. O.: Rheumatoid arthritis with lung lesions. Thorax 11: 234, 1956.
34. RUBIN, E. H. and LUBLINER, R.: The Hamman–Rich syndrome: Review of the literature and analysis of 15 cases. Medicine (Balt), 36: 397, 1957.
35. SCHECHTER, M. M.: Diffuse interstitial

fibrosis of the lungs. *Amer Rev Tuberc* 63 603, 1953.
36. Silverman J J and Talbot T J Diffuse interstitial pulmonary fibrosis camouflaged by hypermetabolism and cardiac failure- Ante-mortem diagnosis with biopsy and catheterization studies. *A Int Med*, 38 1326, 1953.
37 Van Slyck, E. J.: Diffuse interstitial pulmonary fibrosis (Hamman-Rich syndrome) Diagnosis by lung biopsy; treated with cortico-steroids. *Dis Chest*, 31 593, 1957
38 Wildenthal, H. L. and Barclay W R Diffuse interstitial pulmonary fibrosis. *Ann Int Med* 43 1127 1955
39 Wilson M. G and Mikity V G A new form of respiratory disease in prematures. *Amer J Dis Child*, 99 489, 1960.

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CASE REPORT

Goitrous Hypothyroidism Simulating Thyrotoxicosis

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The following case is reported in order to draw attention to diagnostic difficulties in the case of a boy who developed a goitre and was said to be unduly nervous. The results of medical treatment, once the diagnosis of hypothyroidism was est b-lished, were impressive.

Case report

A boy of 6 was admitted to another hospital in November 1954, with a month history of swelling f the neck. His maternal cousin and aunt were said to have had similar swellings and partial thyroidectomy had been performed in each case. The family diet did not suggest a deficiency of iodine. The early life of the patient had been un eventful and he had passed his milestones normally. H was of verage intelligence. H was found t have radio-iodine uptake of 90 per cent with a 24-hour excretion of 10 per cent, which was considered in f vour of thyrotoxicosis.

H was treated with iodine for month without improvement; the dose was doubled for further month, but again there was no improvement. H was then given phenobar bitone which was stopped when he became ery excitable. In July 1955, he began a two-month course of propylthiouracil 50 mg three times daily. His goitre and nervousness increased. A thyroid murmur was said t have been heard and he had a tremor and moist palms, but no eye signs. There was no history of weight loss and bowel action was normal. It was decided to refer him for another opinion as a case of thyrotoxicosis not responding to treatment.

Antithyroid treatment was stopped at the time f admission to the Hospital for Sick Children in September 1955, when he had a smooth, moderately firm goitre with a neck circumference of 34.8 cm (Figure 1). There was no tremor or sweating. B.P 100 70. Pulse 80–110. Sleeping pulse 60–90

Investigations. Serum cholesterol 232 mg/100 ml. B.M.R. 103 per cent of normal for height and weight and 116 per cent for age. His bone age was 8 years.

It was concluded that there was no evidence of thyrotoxicosis and that he might be suffering from an enzyme defect which impaired his ability to synthesize thyroid hormone. H was discharged to be observed without treatment.

H attended monthly in the next five months when his neck appeared t decrease in size. He grew and gained weight slowly.

H was readmitted in April, 1956 for reassessment. His neck circumference had decreased by 2.2 cm since his previous admission, but there were some nodules palpable t the right apex of the thyroid. There was no tremor or sweating. B.P 120 60. Pulse 80–120

Investigations. Serum cholesterol 120 mg/100 ml. Haemoglobin 86 per cent.

It was concluded that no treatment was indicated and he was discharged.

During the next six months he developed a tremor and moist palms. H gained 1.35



Fig 1 Patient in 1955, ged 9 fter 10 months f antithyroid treatment.

kg in weight but was reported to be exci table. H was readmitted in December 1956 for further in vestigation. His neck circum ference had increased to 34 cm and there was no change in the consistency of his thyroid gland. He still had a tremor and moist palms. No thyroid murmur was heard and there were no eye signs. B P 110/80. Pulse 90–1*0 Sleeping pulse 70–90

I vestigations Haemoglobin 94 per cent Serum cholesterol 185 mg/100 ml B.M.R. 128 per cent of normal for height and weight and 139 per cent for age. Chest X ray showed normal lung fields. There was a soft tissue swelling in the neck and the trachea was displaced and compressed. Because of the raised B.M.R he was thought to be thyrotoxic.

Antithyroid treatment was restarted with potassium perchlorat 200 mg twice daily on the 8th January and, on the 22nd January thyroid 8 mg twice daily was added, and he was discharged on this therapy On the 26th January his B.M.R. was 112 per cent of normal for height, weight and age. By discharge on the 29th January his neck circumference was 33 cm.

In March, his neck circumference had in creased to 35.5 cm and he still had tremor and moist palms. His voice had become hoarse There were no eye signs. The potassium perchlorate was stopped and the thyroid continued for a further two weeks. Six weeks later there was no change and he was put on Lugol's iodine 0 3 ml three times daily which was increased by 0.06 ml daily to 0 6 ml three times daily He was unchanged again in September and the Lugol s iodine was reduced to 0 15 ml twice daily In November his neck circumference had increased to 36.5 cm and treatment was stopped. By July 1958, his neck circumference was 36.5 cm. Since his discharge he had gained weight steadily He was started on thyroid 32.5 mg three times daily in an attempt to suppress his T.S.H. (thyroid stimulating hormone). A month later his neck circumference was 34 cm and he had gained almost 0.5 kg in weight. In October his neck circumference was 33 cm and his pulse 84 The improvement in his goitre was maintained when he was seen in March, 1959 His pulse was 88 and he had gained 0.9 kg in six months. H was not sweating

It was felt that this response to thyroid was good evidence that he was unable to manufacture thyroid hormone in adequate quantities and that his goitre resulted from excessive T.S.H. It was decided to stop all treatment and to readmit him at least three months later for investigations to settle this point.

He was admitted in September 1959 aged 13, and was found to be a calm, cheerful, co-operative boy. Bowel action had been normal and there had been no symptoms which might have been attributed to obstruction due to his enlarged thyroid gland. H. was in a Secondary Modern School where his educational attainments were satisfactory. His neck circumference was 36.. cm. He was below the 10th percentile for height and below the 25th for weight. His colour was satisfactory. B.P. 126/80. Pulse and sleeping pulse 80–110. Apart from some nodules at the right apex of his thyroid, there was uniform soft enlargement. No thyroid murmur was heard and he did not have eye signs. His palms were moist, but not warm. There was no tremor. His skin was of normal texture. There was no sign of secondary sex characters.

Investigations. Haemoglobin 94 per cent. Serum cholesterol 175 mg/100 ml. Calcium 10.1 mg/100 ml. Phosphorus 4.0 mg/100 ml. Alkaline phosphatase 16 units/100 ml. Blood sugar curve, fasting was 105 mg/100 ml, ½ hour — 143 mg/100 ml, 1 hour — 128 mg/100 ml, 1½ hours — 121 mg/100 ml, 2 hours — 116 mg/100 ml, 2½ hours 116 mg/100 ml. Twenty-four-hour urinary excretion of creatinine 875 mg and of calcium 58 mg. B.M.R. 120 per cent of normal for age. 106 per cent when referred to creatinine standards of Talbot *et al.* (8). X-ray of wrists for bone age 11–12 years. P.B.I. 2.9 mg/100 ml. Radio-iodine uptake 91 per cent in 5½ hours, radio-activity not discharged by oral potassium perchlorate (400 mg). Renal iodide clearance using radio-iodine 32 ml/min. (Normal 30–35 ml/min.) The following stable iodine estimations were performed by Dr. E. Shalom of University College Hospital Medical School using an unpublished technique. Butanol extractable iodine (B.E.I.) 2.3 mg per cent. Plasma iodine 1.3 microg per cent (0.. microg per cent lower limit of normal). Urine iodide 93 microg/24 hours. (Normal 100–150 microg/24 hours.) The latter two samples were taken at different times and were therefore unsuitable for calculating the renal iodide clearance. Urine organic iodine 8 microg/24 hours. (Normal up to 10 per cent of total iodide.) Plasma iodotyrosines and butanol non-extractable iodine not detected.

The P.B.I. and the B.E.I. performed in different laboratories were subnormal, thereby confirming the suspicion of hypothyroidism. (The former was 0.4 mg per cent less than the latter, which is theoretically impossible, but the difference is within the limits of usual variability.) H. was restarted on thyroid on the 2nd of October and this was increased until he was receiving 260 mg daily by the 20th of October. H. was discharged on this dose when his neck circumference was already reduced to 33.6 cm.

In March, 1960 his neck measurement was 33 cm (Figure 2). He had gained 1.35 kg and grown 2.9 cm since restarting thyroid.

Discussion

Four specific defects in the utilization of iodine for the formation of thyroxine had been distinguished by Stanbury *et al.* (1,7). All are characterized by a high and rapid iodine uptake, which is unaffected in the latter three groups by an oral dose of potassium perchlorate.

a) *Failure to Organify Iodine*

These patients are unable to combine iodine with organic molecules so that a large dose of potassium perchlorate causes the immediate release of iodine from the gland. Direct assay on thyroid biopsies in such a case has confirmed the presence of inorganic iodine (6). Stanbury suggests that the defect is an absence of oxidase in



Fig. 2. Patient aged 13 five months after starting 60 thyroid daily

the thyroid cells. However Haddad & Sidbury (3) consider that an iodinase defect and an abnormal receptor protein might produce the same picture

b) *Failure of De iodination of Iodotyrosines*

Absence of de-iodinase both in the thyroid and the peripheral tissues leads to the escape of mono and di iodotyrosines into the blood and from the body. The hypothyroid state is considered to result from the loss of these hormonal precursors (7)

This concept of a pure de iodinase defect producing hypothyroidism has been criticised by Gardiner *et al.* (2) who suggest that there must be an associated defect in synthesis or release of thyroxine

c) *Failure of Coupling of Iodotyrosines*

Stanbury *et al* (6) describes a patient in whom thyroxine is found in the blood and thyroid gland in subnormal quantities. Large quantities of mono and di iodotyrosines are found in a thyroid biopsy. Werner *et al* (9) also feel that a failure of coupling may explain the defect in hormone synthesis in their case where they found large quantities of mono and di-iodotyrosines in the blood and thyroid gland with normal de iodinase activity of thyroidal and peripheral tissues.

d) *Formation of Abnormal Iodoproteins*

The formation of metabolically inactive iodinated proteins may be associated with hypothyroidism (1). In such cases the P.B.I. may be high and it is the B.E.I which is the more reliable index of the thyroid state

In the present case the data available enables three of these four defects to be confidently excluded. The gland radioactivity at the height of its uptake was not discharged by an oral dose of potas-

sium perchlorate (400 mg) as would have been the case if there was a defect in the organification or iodine The fate of labelled iodotyrosine was not determined, but the failure to detect iodotyrosine in the plasma and the urinary excretion of normal amounts of iodinated organic components makes the de-iodinase type of defect very unlikely Iodine was not detected in the plasma fraction which was protein precipitable but not butanol extractable and this excludes the defects associated with abnormal iodoprotein synthesis

The possibility remains that this case may be similar to that described by Stanbury *et al* (6) where the defect was attributed to a failure of coupling of iodotyrosines. The similarity would be established by demonstrating a raised iodotyrosine content of a thyroid biopsy but biopsy was not undertaken. Certainly the case differs from that of Werner *et al* (9) in that iodotyrosines were not found in the plasma.

The majority of cases of enzyme defects in the synthesis of thyroid hormone which have been reported, have been characterized by obvious clinical features of cretinism. Though the case reported here would be classified as cretin by Stanbury (7) using as his definition permanent retardation in the development of the skeleton or C.N S resulting from thyroid deficiency which existed during foetal or early neonatal life it possessed f w of the clinical features of cretinism and in such a case the description goitrous hypothyroidism is more helpful to clinicians than familial or sporadic cretinism.

That this case was regarded for four years as one of thyrotoxicosis wa partly due to an apparently raised B.M.R illustrating the unreliability in childhood of B.M.R.s related to age Use of the creatinine standards of Talbot *et al.* (8) led to the calculation of a B.M.R which was a more accurate index of the thyroid state A reminder is provided that thyrotoxicosis is not the sole cause of a raised radio-iodine uptake

The results of the treatment of the goitre have been very satisfactory and this is undoubtedly due to the large doses of thyroid which have been used Thyroid surgery may not now be required for cosmetic purposes, but this would be indicated in the event of any increase in the size of the apical thyroid nodules, because of the possibility of malignant change Hubble used 200 mg of thyroid daily in treating a male patient of the same age and had equally good results but the patient of Stanbury *et al.* (6) was treated intermittently in her teens with smaller doses of thyroid, viz. 100 mg and her thyroid enlarged progressively presumably due to failure to remove the stimulus to T.S.H production. In March 1960 his neck measurement was 33 cm (Fig 2) He had gained 1.35 kg and grown _0 cm since restarting thyroid. This weight gain and growth was maintained when he was seen in December 1960 His neck then measured 3_4 cm.

S mmary

A case of goitrous hypothyroidism in a boy of 13 is described which has been successfully treated with "60 mg of thyroid daily The defect in this case may have been due to a failure in the coupling of iodotyrosines For four years the patient



Fig. — P tient aged 13 five months fter starting *60 thyroid daily

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This concept of a pure de iodinase defect producing hypothyroidism has been criti cised by Gardner *et al* ( ) who suggest that there must be an associated defect in synthesis or release of thyroxine

c) *Failure of Coupling of Iodotyrosines*

Stanbury *et al.* (6) describes a patient in whom thyroxine is found in the blood and thyroid gland in subnormal quantities. Large quantities of mono and di-iodotyrosines are found in a thyroid biopsy Werner *et al* (9) also feel that a failure of coupling may explain the defect in hormone synthesis in their case where they found large quantities of mono and di-iodotyrosines in the blood and thyroid gland with normal de iodinase activity of thyroidal and peripheral tissues.

d) *Formation of Abnormal Iodoproteins*

The formation of met bolically inactive iodinated proteins may be associated with hypothyroidism (1) In such cases the P B I may be high and it is the B.E.I. which is the more reliable index of the thyroid state

In the present case the data available enables three of these four d fects to be confidently excluded. The gland radio-activity at the height of its uptake was not discharged by an oral dose of potas-

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CASE REPORT

Leydig Cell Tumour of the Testis in a 6½ Year Old Boy

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Leydig cell tumour is a rare condition, especially in children. About 30 cases have hitherto been reported. Bishop *et al.* have recently reviewed 26 cases (1). Savard *et al.*, in March 1960 (4) reported a case of virilizing tumour in the testis. The tumour had the morphologic appearance of a Leydig cell tumour but biochemical activities were suggestive of an adrenocortical origin. In children the tumour is invariably associated with precocious masculinization. In the present paper the condition is discussed in connection with a case report and with particular reference to urinary steroid excretion.

Case Report

A boy aged 6½ years (Hospital records U.H. 8801) was admitted to the Paediatric Department of Rikshospitalet in January 1960 because of precocious puberty. There had been no known endocrine disturbance in his family. H. was the product of an uncomplicated delivery at term and weighed 3300 g and measured 43 cm. H. developed normally to the age of 2 years. When he started to talk, he was noted to have an unusually deep voice and the voice became even deeper as time proceeded. From the age of 3 years he began to grow unusually rapidly and in particular his penis became larger than normal. Pubic and axillary hair was first noted when he was 4 years old, and at the same time he exhibited acneiform eruptions. There have been no known erections or emissions.

Physical examination on admission revealed a boy with the appearance of a muscular and hirsute youth (Fig. 1). His height of 146 cm was at the 90th percentile for 10 years of age. H. had short lower extremities, measuring from the top of the symphysis to the heels 71 cm, with an upper/lower segment ratio of 1.06 (the average ratio for an 8 year old boy). His weight of 43.9 kg was at the 97.5th percentile for his height. There were pronounced acneiform eruptions, the voice was deep and he had extensive growth of pubic and axillary hair.

The penis was of adult size and proportion, measuring in the nonerectile state 11 cm. The left testis was enlarged, measuring 3 × 2 cm, with a localized firm nodule in the posterior part. The right testis was small, measuring 2 × 1 cm. The blood pressure was 100/70. The patient's mental capacity corresponded to his chronological age, but he was rather shy and reserved.

Laboratory findings. The concentration of Hb. was 14.7 g/100 ml, the leucocyte count 6200/mm³ with 43 percent neutrophiles, 4 band forms, 2 monocytes and 51 lymphocytes. Erythrocyte sedimentation rate was 5 mm/h. Repeated routine urinalyses were normal. The serum concentration of potassium, sodium and nitrogen urea were also normal. The results of hormone analyses on 24 hours urine are shown in the table. Roentgenograms of the skull, chest and skeletal systems were normal, except for a bone



Fig 1 The patient prior to the operation together with a normal boy of the same ge

age corresponding to about 17 years. Pyelograms were normal and there were no roentgenologic signs of adrenal tumour An electroencephalogram was normal. The administration of 100 mg cortisone daily for ten days did not diminish the size of the left testis, and had no influence on the amount of 17 ketosteroids in the urine A biopsy from the firm nodule in the left testis revealed a considerable proliferation of interstitial cells, interpreted by the pathologist as Leydig cell tumour or hyperplasia.

Based on the above the patient was considered to have a Leydig cell tumour and an extirpation was performed F bruary 1 1960. During the operation a biopsy specimen was taken from the right testis.

The gross specimen revealed a testis measuring 3.5 ...5 cm. The cut surface showed a central area of grayish brown colour with a sharp boundary to normal testis parenchyma (Fig). Microscopically the central area consisted of relatively uniform, large polyhedral cells with eosinophilic granular cytoplasm containing lipoid vacuoles. There were no crystalloids present. The nuclei were round, slightly eccentric and regular The tumour had no capsule but was learly demarcated. Scattered trophic testi-



F g 2. The gross specimen showing the cut surface of the testis, with central, grayish brown area and the rrow pointing t the borderline between this and normal testicular parenchyma. The epidid mis and the spermatic cord t the right

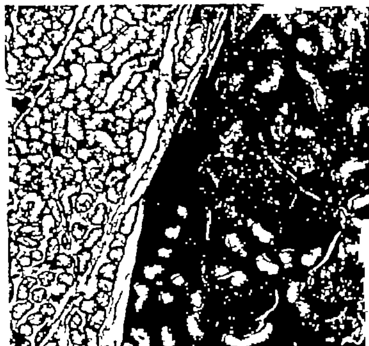


Fig. 3. Low magnification picture showing the clearly demarcated tumour tissue t the right with scattered atrophic testicular tubules in the tumour parenchyma. Normal testis tissue to the left.

cular tubules were seen in the tumour parenchyma.

The testicular tissue outside the tumour showed normal tubules with spermatogonies, spermatocytes, but no mature sperms. Interstitial cells were neither observed in these areas nor in the biopsy from the right testis.

Follow up: Three months later m M y 1960, he was re-admitted for follow up. There were no demonstrable alteration in his signs of precocit. The right testis had grown remarkably in size measuring 4 cm.

Discussion

Cases of precocious sexual development may be classified as complete or incomplete. *The complete type* involves an increased amount of gonadotrophins. The sex hormones are secreted in amounts normal for adolescents and in boys the testes are fully matured. Diseases of the central nervous system are the underlying cause of this condition.

In cases of *the incomplete type* there is precocious development of secondary sex characters without increased amounts of gonadotrophins. Diseases of the adrenal cortex or of the gonads are the cause.

The clinical picture in the present case together with the fact that the gonadotrophins in all but one analysis were less than 6 units per day permitted classification of the patient as an incomplet type of precocious sexual development (macrogenitosomia praecox).

Accordingly the possible diagnoses were

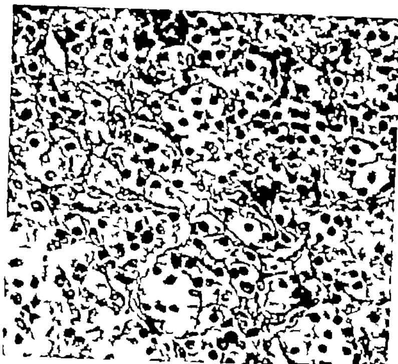


Fig. 4. High magnification view of the tumour: large uniform and polyhedral cells with granular eosinophilic cytoplasm and lipoid vacuoles along with round, slightly eccentric and regular nuclei.

either adrenocortical hyperfunction (tumour or hyperplasia) or interstitial cell hyperfunction (Leydig cell tumour or hyperplasia). The patient had an enlarged left testis and there were no roentgenologic evidence of tumour in the adrenals. The enlargement of the left testis might then depend on interstitial cell proliferation or on abberrant adreno-cortical tissue. Lack of response to a course of cortisone and a biopsy solved this problem.

Hormone analyses may be of much help. The estimation of total 17 ketosteroids in the urine is of limited aid in establishing the cause of macrogenitosomia praecox. Remarkably high figures are likely to be due to neoplasm either of the adrenal cortex or of the Leydig cell. According to Bishop *et al.* the urinary output of 17 ketosteroids have been reported in only 11 of the 26 cases of Leydig cell tumour in children. The findings were of little significance in most of the cases as were the low figures in our case.

Congenital virilizing adreno-cortical hyperplasia is thought to be due to a faulty synthesis of Compound F. Butler & Marrian described in 1937 the presence of pregnanetriol in the urine of two women with the adrenogenital syndrome (2). This metabolite has later been demonstrated in greatly increased amounts in many patients with adreno-cortical hyperplasia. Pregnanetriol has also recently been demonstrated in urine from patients with adreno-cortical tumour. The conclusion seems justified from the literature that a normal figure for pregnanetriol (0–0.5

TABLE 1. *24-hr excretion of hormones and their metabolites in the urine of J. H.*

	Nov. 59	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Gonadotrophins M.U.	<4?-6	<6	<6	<6	<2	Positive	<?
17-KGS mg	11.1	8.1	4.3	7.	1.1	4.9	3.4
17-KS mg	12.8	3.4	4.5	6.9		3.3	3.2
Pregnanetriol mg					0.3	0	
Pregnanediol				1.7	0.3		2
Fractionated 17-KS							
Androsterone mg					1.1	1.0	1.3
Dehydroepiandrosterone mg					0.6	0.2	0.5
Aetiocholanolone mg					0.4	0.6	0.3

mg) in the urine for a patient with sexual precocity serves to exclude the possibility of the usual type of adreno-cortical hyperplasia (without hypertension). Except for the case of Savard et al. (4) the urinary pregnanetriol level has not been reported in a case of Leydig cell tumour despite this probably being the most important hormone analysis for the differentiation between Leydig cell tumour and adreno-cortical hyperplasia. Savard et al. demonstrated in their case increased urinary output of pregnanetriol. The tumour also had in other respects activities suggestive of adreno-cortical properties. A cortisone course was not attempted. The daily excretion of 0.3 mg pregnanetriol in our case was not compatible with a diagnosis of abberant adreno-cortical tissue causing the sexual precocity.

It is universally accepted that Leydig cells produce testosterone. The metabolites of this hormone excreted in the urine are mainly aetiocholanolone and androsterone but the same two substances may also be metabolites of adreno-cortical hormones. Dehydroepiandrosterone which in normal urine constitutes 10-30 per cent of the total 17-ketosteroids is usually considered to be mainly a metabolite of adreno-cortical androgens. The substance gives a strongly positive Allen Blue Test in most cases of adreno-cortical tumour and may be weakly positive in adreno-cortical hyperplasia. Cook et al. applied the micro-chromatographic fractionation procedure of Zygmuntowicz to the urine from a case of Leydig cell tumour in a 5 year old boy (3). Stanbitz et al. (5) and Bishop et al. (1) fractionated the urinary 17-ketosteroids from three cases of Leydig cell tumour. A preponderance of aetiocholanolone and androsterone together with a decreased amount of dehydroepiandrosterone was present in these four cases. In the case reported by Savard et al. there was an increase of the alphaketosteroids (aetiocholanolone and androsterone) as well as dehydroepiandrosterone (and 11 oxygenated 17-ketosteroids). In our case we were not able to demonstrate an increased amount of the 17-alphaketosteroids but the method is probably of limited value for patients with a low total 17-ketosteroid output.

Summary

A case of Leydig cell tumour in a 6½ year-old boy is presented. The need for more elaborate methods for the biochem-

cal separation of Leydig cell tumour from aberrant adreno-cortical tissue is stressed. Normal urinary output of pregnanetriol and a lack of increase of the 17 beta ketosteroids in the urine in a patient with a tumour in one of the testes serve to exclude the possibility of an adreno-cortical disorder

Addendum

Follow up: One year later in February 1961 there still is no demonstrable alteration in the signs of precocity The right testis is about the same size as in May 1960.

References

1 Bishop P M., van Meurs, D P., Willcox, D R. and Arnold D.: Interstitial-cell tumour of the testis in a child. *Brit Med J* *5168* 228–24., 1960.

2. Butler, G. C. and Marrian G F.: Chemical studies on adreno-genital syndrome; isolation of 3 (alfa) hydroxyetiocholane-17 one (isoandrosterone) and new triol from the urine of women with adrenal hyperplasia. *J Biol Chem* *124* 237 247 1960.

3. Cook, C. D., Gross, R. E., Landing, B. H. and Zygmuntowicz A. S.: Interstitial cell tumour of the testis. Study of 5-year old boy with precocious puberty *J Clin Endocr* *12*. 725–736 1952.

4 Savard K., Dorfman R. I., Baggett, B Fielding, L. L., Engel, L. L., McPherson, H. T., Lister, L. M., Johnson D. S., Hamblen E. C. and Engel, F L.: Clinical morphological and biochemical studies of virilising tumor of the testis. *J Clin Invest* *39* 534–551 1960.

5. Stauritz, W J Oberhelmacher, O. J and Black, M. S.: Precocious puberty in case of bilateral interstitial cell tumor of testes. *J Urol*, *65* 562–570, April 1951.

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CASE REPORT

Premature Twins with Esophageal Atresia and Tracheo-esophageal Fistula

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Esophageal atresia occurs approximately once in four thousand births. It rarely occurs in one member of twin siblings. Approximately 18 such infants are reported in the English literature (1) Six of these twins were "thought to be identical" To our knowledge, esophageal atresia occurring in both members of a pair of twins has not been previously reported (1 2, 3 4). This paper is the report of a pair of male twins, both of whom were born with esophageal atresia and tracheo-esophageal fistula, and both of whom survived surgical correction in spite of advanced prematurity Because these twins are of the same sex, have a similar appearance have identical anomalies and identical serologic phenotypes, they are believed to be genetically identical.

Case Report

The twins were born on January 4, 1960, to 33-year-old gravida III, para I abortion I Caucasian mother Estimated duration of gestation was 34 weeks. Pregnancy was complicated by minimal vaginal bleeding during the third month. This responded to restriction of physical activity During the third trimester there was an abnormally rapid increase in abdominal girth. At delivery this proved to be due to a marked excess of amniotic fluid. This is a common finding in mothers who give birth to infants with esophageal atresia because the amniotic fluid cannot reach the fetal intestinal tract for reabsorption (8, 9)

The mother and father each had a child or children by a previous marriage. None of these half-siblings had congenital defects. The twins were the first infants of this marital union. The mother and father have no congenital defects and none are known in relatives of either

At birth infant A weighed 1672 g Infant B weighed 1532 g There was a single placenta with two umbilical cords originating adjacent to each other near the center of the placenta. There were two amniotic sacs. The placenta and membranes were not examined for separate or common chorions. Identical twins may have individual amniotic sacs but do not have individual chorionic envelopes (5)

Both twins were vigorous and no physical abnormalities were noted at birth. On the third day of life, feedings were attempted and were promptly regurgitated. X rays revealed esophageal atresia and tracheo-esophageal fistula in each infant (Fig 1) Pharyngeal suction was started immediately and surgical therapy planned. This was performed in a staged manner as has been described in a previous communication (10) The mortality



Fig 1 X-ray of twin A on the left and twin B on the right. Radiopaque dye has been placed in the esophageal pouch which ends blindly at the third dorsal vertebra. Notice gas in the abdominal viscera indicative of associated tracheo-esophageal fistula. The gas passes from the trachea through the lower esophageal segment int the stomach and intestine.

rat in premature infants with esophageal tresia has been unacceptably high when an anastomosis between the two esophageal segments has been performed as a primary definitive procedure We now routinely treat premature infants by a staged regimen.

Course of Inf nt A

Infant A weighed 144 g at age three days, when a Stamm gastrostomy was per formed under local anesthesia (Figs. 2 and 3) Following this the infant continued t be vigorous, and twel hours later under general endotracheal anesthesia the tracheo-esophageal fist la was doubly ligated This procedure was performed extrapleurally through the bed f the fifth right rib (Fig 4) The infant could then be fed by gastrostomy without fear of regurgitation of feedings into the trachea (Fig 5) A special nurse was in constant attendance to insure complete removal of pharyngeal secretions.

On January 3, 1960, fifteen days after ligation of the fistula there was evidence of formula leaking from the stomach through the esophagus and through the fistula into the trachea. The tracheo-esophageal fistula had reopened. Feedings were discontinued and tracheal suction performed to correct the resultant aspiration pneumonitis. The patient had gained 420 g fifteen days, now weighing 1872 g The following day after much of the aspiration pneumonitis had been corrected, the third surgical procedure was performed. Through a transpleural right thoracotomy the tracheo-esophageal fistula was divided and an end to-end esophageal anastomosis performed (Fig. 6 and 7). Post

operatively the infant developed right upper lobe atelectasis which was corrected by endotracheal suction. The baby was fed by gastrostomy for two weeks at which time small oral feedings were started. These feedings were gradually increased until full feedings were being taken by mouth without difficulty. Weight gain was satisfactory and on February 24, 1960 31 days after the esophageal repair and at age 51 days, the infant was discharged from the hospital weighing 2.76 kg. He has continued to gain weight and at age eleven months weighs 8 kg (Fig. 8)

Course of Infant B

Infant B weighed 1312 g at age three days and was treated in a similar fashion. A Stamm gastrostomy was performed under local anesthesia as soon as the diagnosis had been made (Figs. 2 and 3). The patient tolerated this procedure well and remained vigorous. Twelve hours later the tracheo-esophageal fistula was doubly ligated by the extrapleural approach through the bed of the right fifth rib posteriorly (Fig. 4). A small opening was made in the pleura during the dissection, and postoperatively the infant developed a pneumothorax requiring tube thoracostomy. The following day gastrostomy feedings were started and a steady weight gain ensued. Since infant A had had leakage from the fistula at age fourteen days, methylene blue was added to the formula of infant B so that a leak could be identified promptly thus minimizing aspiration pneumonitis. At age 16 days the methylene blue appeared in the pharyngeal suction, therefore the gastrostomy feedings were stopped, and there was no evidence of

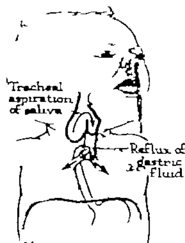


Fig. 2. The lungs are insulted from two sources. Saliva spills over from the upper esophageal pouch and gastric contents reflux into the trachea through the tracheo-esophageal fistula. (Used with permission from *Western J Surg* WOOLLEY M. M.: The premature infant with esophageal atresia.)

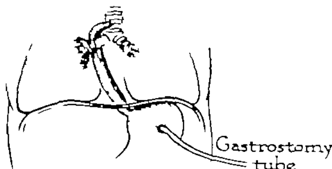


Fig. 3. A Stamm gastrostomy is performed under local anesthesia for subsequent gastrostomy feedings. (Used with permission from *Western J Surg*, idem.)

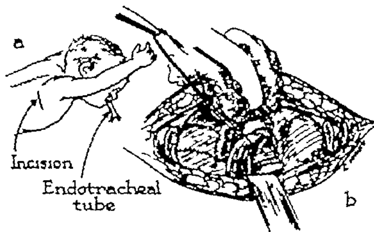


Fig. 4

aspiration pneumonitis. The infant weighed 1895 g, a gain of 563 g in 2* days. The fol lowing day the tracheo-esophageal fistula was divided, and an end to-end esophageal anastomosis was performed transpleurally (Fig 6 and 7) Gastrostomy feedings were continued postoperatively and oral feedings were started in small amounts and gradually

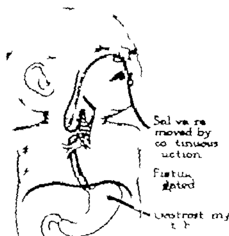


Fig. 5 Salivary secretions are removed from the upper pouch by catheter. The infant is fed through the gastrostom tube without fear of reflux of feedings int the trachea since the tracheo-esophageal fistula has been ligated.

increased. Six weeks after the anastomosis the baby developed progressive difficulty with oral feedings, therefore a Dionosil (R) swallow was performed which showed a very tight esophageal stricture as well as recurrence of the tracheo-esophageal fistula (Fig. 9) On March 3 1960, at age 69 days, the infant underwent the third thoracotomy The fistula had recurred at the anastomotic site and there was also a tight stricture of the esophagus with only a pinpoint lumen remaining The fistula was reclosed, the stricture resected, and an end-to-end esophageal anastomosis performed. The infant again required endotracheal aspiration on several occasions postoperatively but progressed from gastrostomy feedings to oral feedings without difficulty The weight gain was steady and the infant was discharged from the hospital on March 25, 1960, at age 81 days weighing 3.06 kg All feedings were being given by mouth, his gastrostomy tube having been removed Weight gain was progressive and there was no dysphagia until age five and one-half months at which time a single esophageal dilatation was required. This relieved both the dysphagia and wheezing which was due to pressure of the upper esophageal segment on the trachea. The infant's weight at eleven months was 8 kg (Fig 8).

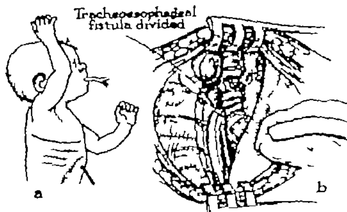


Fig. 6. (a) A long incision is made through the fourth intercostal space. The pleura is opened widely for good exposure. This is simpler than the extrapleural route. (b) The tracheo-esophageal fistula is divided, the previously placed ligature being visualized. (Used with permission of *Western J Surg idem.*)

Discussion

Haight (1957) reported a set of twins who were thought to be genetically identical, but only one of whom had esophageal atresia (1). The embryological reason for this is that monozygotic twins have their origin within two weeks of fertilization (6). The lung bud does not make its appearance until two weeks later at approximately four weeks after gestation. Still later the trachea and esophagus develop by septation (6, 7). It becomes apparent then that although twins may be genetically identical, that is, originating from one fertilized ovum, one of the twins may have esophageal atresia because of subsequent development in only one of the embryos. This is given as the reason for esophageal atresia not having occurred in both members of a set of identical twins. In other words, this may be embryologically a developmental defect rather than a genetically transmitted one. It would seem entirely possible, however, that this defect could be transmitted in a genetic sense to each member of a pair of identical twins. This case report presents such a possibility. These twins are of the same sex (male), they have a similar appearance, they have an identical anomaly, and their blood types are identical (Table 1).

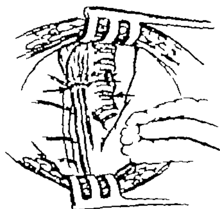


Fig. 7. The arrows indicate the esophageal anastomosis and the site of closure of the tracheo-esophageal fistula. (Used with permission of *Western J Surg, idem.*)



Fig. 8. Thi is photograph of the twins t age eleven months. Twin A is on the right and twin B is on the left



Fig. 9 X ray of twin B Dye was placed in the esophagus and immediately passed through a recurrent tracheo-esophageal fistula into the trachea and bronchi Notice that no dye has passed through ery tight stricture mt the distal esophagus.

TABLE 1 *Serological types of the mother and father and twins*

	Mother	F ther	Twin A	Twin B
Type	O	A	A	A
Rh	+	+	+	+
Kell	Neg	Neg.	Neg.	Neg
Duffy	Neg.	Pos.	Pos.	Pos.
MN	NN	MN	MN	MN
CDE	CDE/ce	DE/ce	CDE/ce	CDE/ce

It will be noted that type A was contributed by the father who may or may not have been homozygous for this characteristic Both parents and infants are Kell negative so that this blood factor contributes no information to the question of the twins identity They both inherited the Duffy positive factor from their father and they must both have inherited the M factor from their father who was heterozygous for it since their mother was N The Rh types can be treated in terms of most probable genotypes from the test

results these suggest that both the father and the mother were heterozygous at the Rh locus and that both twins inherited the same allele from the heterozygous mother and the same allele from the heterozygous father. These results permit a statistical deduction that the probability that these twins are fraternal is less than .05. This suggestion, that they are in fact identical, is derived from a statistical analysis of the serologic types alone and does not take into account the supporting evidence of their appearance, sex, or any other more subjective findings.

In an analysis of deaths due to esophageal atresia it is found that the smaller the infant the less chance there is for his survival, if a primary esophageal anastomosis is performed. We have found that staged therapy for premature infants provides a much better chance for survival. Simple ligation of the tracheo-esophageal fistula is the quickest and easiest way of interrupting the fistula so that the infant can then be fed by gastrostomy. When he weighs more and is more mature he is a better surgical risk and a definitive correction can be performed at that time.

Recurrence of the tracheo-esophageal fistula, which occurred in twin B, is an uncommon complication occurring in approximately 1% of these infants. This complication is probably due to an infection at the anastomotic site which drains simultaneously into the trachea and esophagus, thus reforming the fistula. There was no evidence of infection at the anastomotic site in this infant, but it could have cleared by the time surgery was performed.

Summary

To our knowledge this is the first reported case of esophageal atresia occurring in both members of a pair of twins. These twins appear to be genetically identical because of their general appearance, sex, identical anomaly, and identical serologic genotypes. Genetic predisposition for esophageal atresia in twins would seem to be possible from this study. Heretofore it has been thought that esophageal atresia in one of identical twins has been a developmental defect rather than a genetically transmitted one. Staged therapy for the premature infant with esophageal atresia increases the chance of survival of these infants.

Acknowledgements

The statistical analysis of the serological types was performed by Ray Owen, Ph. D., Professor of Biology, California Institute of Technology, Pasadena, California.

References

1. Haight, C.: Some observations on esophageal atresias and tracheo-esophageal fistulas of congenital origin. J. Thor. Surg. 34: 141, 17–, 1957.
2. — Personal communication.
3. Potts, W.: Personal communication.
4. Gross, R. E.: Surgery of Infancy and Childhood. Philadelphia and London, W. B. Saunders & Co., 1953.
5. Gross, R. E. and Woolley, M. M.: Esophageal atresia and tracheo-esophageal fistula: analysis of 100 consecutive cases. (Unpublished.)
6. Arey, L. B.: Developmental Anatomy. A Text Book and Laboratory Manual of Embryology. Philadelphia and London, W. B. Saunders & Co., 1946.
7. Nelson, O. E.: Comparative Embryology

of the Vertebrates. New York, The Blakiston Co., Inc., 1933.
8. BARKER, J. L.: Congenital Anomalies of the Viscera. Boston, Harvard Press, 1957
9. LLOYD J. R. and CLATWORTHY H. W.: Hydramnios as an aid t the early diagnosis of congenital obstruction of the alimentary tract: study of the maternal and fetal factors. *Pediatrics*, *21* 6: 903–909, 1958.
10. SCOTT J. S. and WILSON J. K.: Hydramnios as an early sign of esophageal atresia. *Lancet*, 569–572, September 21 1957
11. WOOLLEY M. M.: The premature infant with esophageal atresia. *Western J Surg*, In print.

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REVIEW ARTICLE

Peptic Ulcer in Children

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Peptic ulcer is generally considered to be an unusual disease in childhood. ...ated case reports are to be found in the literature but accounts of any larger ...ries are rare. In 1949 Karlström reported 30 cases diagnosed at 25 paediatric clinics ... Sweden during a period of 5 years (3) His work has been of value in attracting attention to the incidence of peptic ulcer in children. His study did not however ...lude a detailed account of the clinical ...atures of the condition. More recently Ramos et al., presented a series of 32 cases which they analysed from different ...ints of view (4). Several of these cases, however had been first diagnosed as ...lts and had been included in the material because the typical history dated ... childhood.

During recent years a striking number ... children have been treated for peptic ulcer at the Gothenburg Children Hospital. T... obtain some idea of the incidence ...d clinical character of the disease we have studied all cases of peptic ulcer treated here since 1945 This hospital has the ad antage of being the only children s ...spital catering for a well-defined district A special study has been made of those cases which have been under observation for more than 5 years. In order to enlighten certain factors of aetiology a number of the most recent cases have been subjected to special psychiatric-psychological examination.

Incidence Age and Sex Distribution Type of Ulcer

Since 1945 a total of 36 patients with the verified diagnosis "peptic ulcer have been admitted to this hospital on one or more occasions The diagnosis was confirmed by X ray in 35 patients, and at operation in the remaining one. The number of new cases from the hospital s admission district for the last three 5-year periods were 4, 10 and 17 respectively which demonstrates a definite increase in incidence (Fig 1) This increase cannot be explained by an increase in local population nor probably by improved diagnosis. It is our impression that a true increase has taken place in the incidence of peptic ulcer in children. Among the grounds for this belief is the fact that most patients displayed such typical signs and symptoms that diagnosis was virtually inevitable

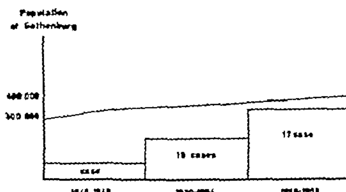


Fig 1 The number of new cases of peptic ulcer discovered at The Gothenburg Children's Hospital during the three last 5-yea periods related to the population served by this hospital during the same periods of time

All patients except one were 9 years or older at the time of first admission (Fig 2) In some it was possible to obtain a typical history dating from the age of 6–7 years The disease appears to be exceptional in the pre-school age group

Our series includes only one such case This was a girl, aged 23 months at the time of admission, in whom ulcer was discovered at operation undertaken because of a 2 week history of increasing vomiting of the obstructi type Laparotomy revealed a little radiating ulceration at the junction between the gastric and duodenal mucosa. Considerable muscle hypertrophy was noted which caused severe stenosis of the pylorus. According to the operation notes, this hyper trophy as not similar t that of congenital hypertrophic pyloric stenosis.

Our material consists of 27 boys and 9 girls. Thirty-one children had duodenal ulcer and 5 had gastric ulcer

Symptoms

Table 1 illustrates the incidence of the different symptoms regardless of the stage in the disease at which each symptom appeared Abdominal pain was present in all but one child A striking number of patients complained of typical delayed pains or admitted relief of pain after food. In 18 patients this typical ulcer history led to the diagnosis Nocturnal pains were also quite a common feature and were of diagnostic significance in 4 cases where pains during the day were

TABLE 1 *Incidence of the different symptoms regardless of when they appeared in the course of the disease*

Pain	35
Delayed pains, relief after food	26
Nocturnal pains	15
Pain related t meals	11
Atypical pains	3
Tenderness on palpation	18
Periodicity	14
Heartburn regurgitation	19
Vomiting	17
Haematemesis	4
Obstructive vomiting	
Vomiting in uncomplicated ulcer cases	11
Haemorrhage	8
Macroscopic	6
Occult	2
Anaemia	5
Anorexia	9
Loss of weight	7
Fatigue	7

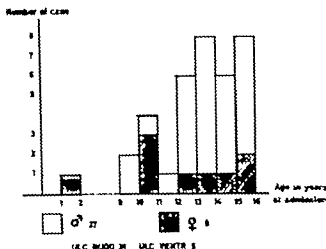


Fig. 2. Age, sex distribution of children with duodenal or gastric (ventr.) ulcer

absent or atypical. Pain directly related to meals could usually be attributed to a certain type of food. In only _ children was this a dominant feature prompting X-ray examination of the stomach. Five patients had atypical pains throughout the course of their disease.

Heartburn and or regurgitation were noted in 10 patients. Histamine test meal was performed in 11 cases; there was hyper secretion in 3, normal findings in 6, while 1 case showed hyposecretion and 1 gastritic values.

In previous descriptions of peptic ulcer in children vomiting and haemorrhage have been given as leading symptoms. In our series only 5 patient with uncomplicated peptic ulcer had repeated vomiting as an important symptom. In these cases vomiting was related to severe abdominal pains and brought about a relief of pain. Obstructive vomiting was seen in patients in one of whom it was the only symptom. Haematemesis occurred in 4 of the 5 patients with macroscopic bleeding and was the reason for admission to the hospital. Occult blood was discovered in 3 children. Five patients were anaemic but in [illegible] no active bleeding could be demonstrated. There was no instance of perforation during the period of study.

Table 2 illustrates the symptoms which were of diagnostic significance in the particular cases. The duration of symptoms up to the time of diagnosis was usually

TABLE * *Predominating and diagnostic symptom at the time of (the first) admission together with the duration of the symptoms prior to admission*

	No. of cases	Duration in years: Under 1	1–2	2–3	Over 3
Typical ulcer history	15	10	2	2	4
Nocturnal pains	4		1	1	2
Pain at mealtimes	2	2			
Atypical pains	5	3	1		1
Haemorrhage	5	2	2		1
Miscellaneous	2	1			1
Total	36	15	6	3	9

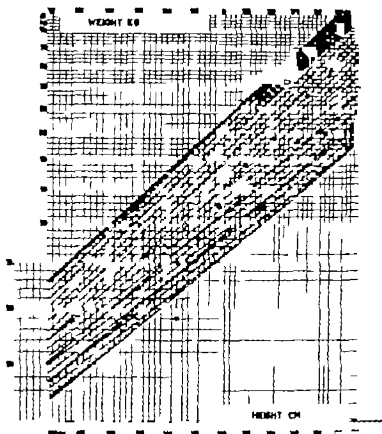


Fig. 2. Height and weight of the boys on (the first) admission to hospital.

clear from the case history and varied from one month to several years. Seventeen patients had a history of less than 6 months. However some had had symptoms for several years and could not state the duration with any precision.

It is striking that many patients gave a quite typical history of the disease. It does not appear to have been difficult to differentiate children with ulcer from those with functional conditions causing abdominal pains. The latter does not seem to be particularly common among children with ulcer. In a few children, vague types of abdominal pain preceded typical ulcer symptoms sometimes for a long period.

Treatment and Prognosis

The great majority of patients quickly became symptom free on the usual regimen of rest, diet and medical treatment. The time taken to achieve healing, as confirmed radiologically was more variable. In some this has not been done.

Three patients were operated upon before they reached the age of 16 because of failure of medical treatment, or repeated recurrences. Eleven of 28 patients had at least one recurrence before 16 years of age.

Follow up was performed in 14 children in whom the observation period from the first hospital admission was more than 5 years (Table 3). Only 2 of these were free

TABLE 3 *Follow-up study of 14 cases which have been under observation for more than 5 years*

One or more relapses confirmed by X-ray	9
Treated by operation	5
Conservative treatment only	4
Typical ulcer symptoms, not necessitating admission or sick leave	3
Symptom-free	2

from symptoms The prognosis can therefore be said to be unfavourable

Aspects of Aetiology

From an aetiological point of view ulcers fall into 2 categories. The first type appears secondarily to some other severe illness Our series contains no certain case of this kind. The other kind of ulcer is a primary chronic disease considered to be psychosomatic in origin (6) This latter type may presumably occur in susceptible individuals under certain external circumstances.

In 25 of our patients (circa 70 %) there was a family history of peptic ulcer usually in one of the parents. This figure is higher than in a series of adults and could suggest that constitutional factors are important in the causation of the lesion (4) We have studied the incidence of certain constitutional features which are considered to be associated with a predisposition to ulcer

The sex distribution has already been discussed. In our series boys were definitely in the majority Fig. 3 illustrates the boys weight and height on admission to hospital. Most of them fall in the area of low body weight in relation to height This could possibly be accepted as leptosom habitus since loss of weight was an unusual and less prominent symptom.

Blood group O was found in 13 of the 21 grouped patients, i.e. in 62 % as against an expected 38 % (1)

Terman–Merrill testing was carried out in 16 patients and the mean I.Q was found to be 110 indicating that children of above-average intelligence are over represented among peptic ulcer patients. A psychiatric opinion was given in 14 cases. Of 11 children, whose psychical constitution could be determined, 6 were considered psycho-asthenic.

The psychiatric examination also included an evaluation of environment. In 6 cases stress factors were discovered (broken homes in 2 cases, threats of divorce long-drawn family dispute alcoholic step-father and psychic strain at school and work). Five patients were considered to have good home environment. The remaining 3 formed an intermediate group

As a rule the patients feeding habits were gone into and irregular or unsatisfactory meals were admitted in only 2 cases. Feeding difficulties in infancy organic abdominal disease or other somatic disturbances did not appear to be common. No patient had had hypertrophic pyloric stenosis in infancy

Projective test methods in children may indicate the existence of significant emotional disturbances. Rorschach testing was performed in 14 cases. The results did not suggest that neuroses or emotional disturbances were common and prominent among children with ulcer (6) All patients tested gave both a high number of answers and whole answers, which is characteristic of high intelligence. But even if

the I Q is taken into consideration, the *number* of answers and whole answers is unusually high. This is usually interpreted as an ambitious attitude These results were in agreement with the clinical im pressions of the patients.

In spite of the limited material it would appear that constitutional factors are important in the causation of peptic ulcer in children. Factors of environment appear to be less obvious. This could be taken as signifying that patients affected with peptic ulcer in childhood comprise a selection of children with particularly strong predisposition to the disease

Summary

A study has been made of 36 children with the established diagnosis peptic ulcer All patients with one exception, were children of school age The following aspects of the disease have come to light

1 The incidence of peptic ulcer among older children appears to be increasing

2. These patients often present a strikingly typical history Delayed pains and/or relief of pain by food were noted in 28 patients. Other typical ulcer symptoms occur commonly

3 Macroscopic bleeding was discovered in 5 cases obstructive vomiting in _. There was no case of perforated ulcer

4. Recurrence was common. Of 14 children observed for a minimum period of 5 years, only _ had been symptom free.

5 Constitutional factors are important in the causation of peptic ulcer in childhood. A high proportion of patients have a family history of the disease Boys are affected more than girls. Patients of blood group O are present in abnormally high proportion Psycho-asthenic constitution is common among these patients.

Environmental factors appear to be less obvious. Neuroses or emotional distur bances were not found to any great extent

Acknowledgement

W are greatly indebted to Mr S. Dworetzky chief psychologist t the Dept of Child Psychi atry who has performed the psychological ex aminations.

References

1 Buckwalter, J A Wohlwend E. B Colter, D C Tidrick, R. T and Knowler, L A Peptic ulceration and ABO blood groups *J.A.M.A* *162* 1215, 1956.

2. Chapman A. H Loeb D G and Young, J B A psychosomatic study of five children with duodenal ulcer *J Pediat* *48* 248, 1956.

3. Karlström, F Ulcus-Krankheiten beim Kinde mit besonderer Berucksichtigung der Häufigkeit. *Helv Paediat Acta*, *4* 455, 1949

4 Ramos, A. R Kirsner, J B and Palmer, W L: Peptic ulcer in children. *Amer J Dis Child*, *99* 135, 1960

5. Sternäck, A. Cardial angestneuros och ulcus duodeni. *Nordisk Psykiatrisk Medlemsblad* *11* 98 1937

6 Westphale, O The peptic ulcer individual *Acta Psychiat Scand Suppl.* *11* 1953.

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A Follow Up Study of Hyperbilirubinaemia in Full term Infants without Iso-immunisation

by J BJURE, G LIDÉN T REINAND and A. VESTBY

The harmful effects of hyperbilirubinaemia in haemolytic disease of the newborn are well documented, and the indications for treatment generally accepted. Exchange transfusion has been recommended when the serum bilirubin level exceeds 18 mg % (11) or 20 mg % (3 5, 7) There is uncertainty however about the deleterious effect of hyperbilirubinaemia in babies with severe neonatal jaundice without evidence of haemolytic disease Practice differs as regards exchange transfusions in these cases. The purpose of this investigation is to elucidate this problem by a follow up study of deeply jaundiced full-term newborns without evidence of haemolytic disease The findings in infants who were treated by exchange transfusion are compared with those in untreated patients.

Material and Methods

The material comprises 113 full-term infants, born at the two maternity hospitals in Gothenburg, during the years 1956 and 1957 In all infants the serum bilirubin level rose to 18 mg % or more The infants were divided into three groups. Group I (uncomplicated cases) comprise babies in whom the pregnancy delivery and neonatal period were completely normal with the exception of hyperbilirubinaemia, Group II (slightly complicated cases) include those in whom there were slight complicating factors in pregnancy or delivery such as simple proteinuria of the mother certain types of medication administered to the mother without obvious effect to the infant, or the umbilical cord loosely around the child's neck, which probably did not affect the infant; Group III (complicated cases) consists of cases with complications which might be deleterious to the child, including asphyxia, abnormal presentations, toxaemia, or surgical intervention. Some infants in all three groups were treated by exchange transfusion, though more in Group III than in the others.

The control series comprises 51 full-term infants born during the same period following normal pregnancies and uncomplicated deliveries and without marked icterus or other complications in the new-born period The whole material is listed in Table 1

A direct Coombs test was performed on all jaundiced infants, and was negative. In 43 an indirect Coombs test was also done; this, too was invariably negative Bilirubin determinations were performed on capillary finger or heel blood by the method of Jendrassik-Grof (8) In order to determine the incidence of severe hyperbilirubin

This investigation was made possible by grant from "Majblomman" Fund

TABLE 1. *Total Number of Children Examined in Different Groups*

Uncompl. cases		Slightly compl. cases		Compl. cases		Controls
No exchange transf.	Exchange transf.	No exchange transf.	Exchange transf.	No exchange transf.	Exchange transf.	
23	4	29	10	29	18	
27		39		47		
66				47		51

aemia during the neonatal period serum bilirubin determinations have been performed during a one year period in all infants with jaundice. Of 3,597 full term infants born at one of the maternity hospitals during 1957 a total of 47 or 1.3% had a bilirubin of 20 mg per 100 ml or more, which is roughly in accordance with a recently published study (9).

The follow up study, which has included physical and neurological examination, as well as Merrill-Palmer testing (15), was performed at an age of 2–3 years. On the slightest deviation from normal the child was subjected to psychiatric examination; this was done in 25 infants. The following features have been taken as symptoms of early brain damage: 1. Hyperactivity, restlessness and inability to concentrate; 2. Stereotypic behavior; 3. Impairment of voluntary movement (clumsiness, fumbling, late motor development); 4. Mental retardation. Since none of these features is pathognomonic of early brain lesion, the diagnosis has been made only where it was impossible to explain the symptoms by other causes, such as environmental factors. Play audiometry was also performed in 123 children, 84 with jaundice and 39 controls.

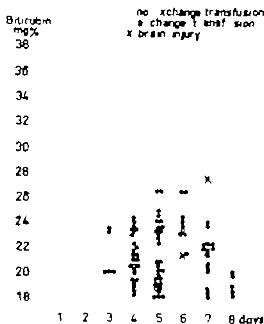


Fig. 1. Maximum bilirubin level in each infant.

Results

No signs of kernicterus were demonstrable during the newborn period. Fig. 1 shows the age when maximal bilirubin levels were found in the different cases, the result being in agreement with what has been previously reported (12). Exchange transfusions were performed on the day of the highest bilirubin level.

On general physical examination no relevant abnormalities were noted. The results of Merrill–Palmer testing are shown in Tables 2 and 3. No difference was found between the groups regarding I.Q. distribution (Table 2). When the material is classified according to the level of hyperbilirubinaemia there is still an apparently normal I.Q. distribution in all groups (Table 3). It can be seen that the distribution of patients with respect to bilirubin concentration is also approximately the same in both groups.

Table 4 shows the number of infants examined by means of play audiometry. No deafness of the central type was revealed.

Table 2 I Q Distribution within Different Groups

IQ	Uncompl. cases: No exchange transf.	Uncompl. cases: Exchange transf.	Slightly compl. cases: No exchange transf.	Slightly compl. cases: Exchange transf.	Compl. cases: No exchange transf.	Compl. cases: Exchange transf.	Controls
150	—	—	—	—	—	—	—
140	—	—	1	—	—	1	—
130	3	1	—	—	1	—	5
120	5	—	4	—	1	2	5
110		1	6	2	9	5	5
100	5	2	9	4	8	3	21
90	4	—	5	3	8	7	10
80	2	—	1	1	1	—	3
70	—	—	3	—	—	—	2
60	—	—	—	—	—	—	—
50	—	—	—	—	1	—	—
Total	23	4	29	10	29	18	51

– Cases with brain injury

Table 3 I Q Distribution in Relation to the Level of Hyperbilirubinaemia

IQ	Uncompl. cases + slightly compl. cases: Bilirubin in mg% 18–19.9	20–24.9	25+	Compl. cases: 18–19.9	20–24.9	25+	Controls
150	—	—	—	—	—	—	—
140	—	1	—	—	1	—	—
130	1	3	—	—	—	1	5
120	2	6	3	2	2	—	5
110	3		—	5	7	2	5
100	5	15	3	1	8	2	21
90	5	6	1	3	7	4	10
80	1	2	1	1	—	—	3
70	—	2	—	—	—	—	2
60	—	—	—	—	—	—	—
50	—	—	—	—	1	—	—
Total	17	42	7	12	26	9	51

– cases with brain injury

The incidence of a suspected brain lesion in the 75 children examined by the child psychiatrist is shown in Table 5. The 3 cases in whom a brain lesion was diagnosed belonged to Group III (complicated cases). One of them had been treated by exchange transfusion. Symptoms suggestive of brain lesion were recognised in 9 children, but in 6 of these the symptoms could be alternatively explained. In the remaining 3 cases the diagnosis of a brain lesion could be made with confidence

TABLE 4. *Cases Examined with Play Audiometry*

Uncompl. cases		Slightly compl. cases		Compl. cases		
No exchange transf.	Exchange transf.	ᵇ exchange transf.	Exchange transf	No exchange transf.	Exchange transf.	Controls
15 (23)	4 (4)	21 (29)	10 (10)	21 (29)	15 (18)	39 (41)

Total number of children within brackets.

TABLE 5 *Psychiatrically Examined Children*

	Uncompl. cases		Slightly compl. cases		Compl. cases			
	No exchange transf	Exchange transf.	No exchange transf.	Exchange transf	No exchange transf.	Exchange transf.	Controls	Total
No of psychiatric exam. children	3	1	4	2	10	3	2	25
Symptoms								
Hyperactivity Restlessness, Lack of concentration.	1	—	—	—	2	1	—	4
Stereotypic behaviour	—	—	1	—	2	—	—	3
Motor disorders	—	—	1	—	1	—	—	2
Mental retardation	1	—	1	—	1	—	—	3

ᵇ = cases with brain injury

Case 1 Boy born 29.8.56, birth weight 3 720 g, second child of a 33 year old woman, whose first pregnancy was in 1940 Except for vomiting during the first months the pregnancy was normal. Delivery occurred after a 2 1/2 hour labour with left occipito-anterior presentation. No signs of asphyxia in utero were present but slight extrauterine asphyxia occurred accompanied by marked petechial haemorrhages on the face. Blood groups: mother O Rh + child O Rh + Coombs direct test negative Icterus was noted with a maximum bilirubin value of 21 8 mg % on the 7th day The child was able to sit at 6 months, walked at 1 year and talked at 21 months. Suspected ECHO infection at 1 year and convulsions in association with fever at 18 months. Somatic examination 22 1 59 showed nothing abnormal. I Q 22.1.59 was found to be 110. Psychiatrist's opinion on examination 9.9.64: "Marked hyperactivity restlessness, lack of concentration and lack of inhibition, which cannot be explained by deficiencies of environment. *Brain* lesion syndrome without intelligence defect."

Comment: Quick labour with considerable variations in pressure congestive haemorrhage.

Case 2 Boy born 3.7.56, birth weight 3 360 g First child of a 34 year old mother who had aborted in the 3rd month of a previous pregnancy in 1955. Proteinuria was noted towards the end of the pregnancy Low forceps delivery occurred following 22 hours labour with the head presenting in left occipito-anterior position. Indications: green liquor and weak pains. The umbilical cord was wound tightly around the neck, on which its impression could be seen. Slight extra-uterine

asphyxia occurred Blood groups: mother A Rh + child A Rh + direct Coombs test negative. Icterus ppeared on the 2nd day and the maximum serum bilirubin level, 21.3 mg %, was recorded on the 6th day Development was retarded, unable to sit at 1 year starting t walk at 2 years. H had repeated respiratory infections during the first year of life Physical examination 26.2.59, revealed nothing bnormal except the retarded development Testing 20.5.59 showed an I.Q of 55. Psychiatric opinion 30.9 59 Difficult to get into contact with, clumsy movements, patient severely retarded, but not restless or lacking concentration. Unfavourable environment. Brain lesion syndrome with mental defect together with environmental injury

Comment. Rather protracted delivery with signs of intra and extra-uterine asphyxia.

Case 3 Boy born 2.7.57 birth weight 3.060 g, first child of 33 year old mother Delivery by breech presentation following a 4 hour labour Blood groups: mother O Rh + child O Rh Direct and indirect Coombs tests negative Icterus developed on the 2nd day with a *maximum bilirubin level* of *7.5 mg % on the 7th day Exchange transfusion was performed. The child could sit without support t the age of 7 months; he walked at 11 months, and formed a few sentences at 26 months. Physical examination 3.9.60 revealed no definit abnormality Testing 23.9.59 showed an I Q 96. Psychiatric examination 24 11.59- Severe restlessness and lack of concentration, contact only superficial and of short duration F vourable environment Brain lesion without intelligence defect

Comment Quick labour breech delivery

Discussion

In a recent publication (12) Mores t al concluded that hyperbilirubinaemia without iso-immunisation does not in olve risk of brain damage to healthy full-term infants though details of the time and nature of follow up examinations were not included. In the same report a follow up survey of premature infants with hyperbilirubinaemia in the absence of iso-immunisation was given. A much lower incidence of kernicterus was found than has been noted by other workers (1 3 4). Exchange transfusion was not performed in either the full-term nor prematur infants reported by Mores *et al* and on the basis of their results they suggested that exchange transfusion was unnecessary in hyperbilirubinaemia, unless iso-immunisation was present.

Experiments have shown that an important factor in the causative mechanism of brain injury due to hyperbilirubinaemia is the functional maturity of the blood brain barrier (6). Prematurity and asphyxial or traumatic injury at the time of labour might impair the blood brain bar rier and thus increase the risk of a brain lesion due to hyperbilirubinaemia. Over dosage of such vitamin K analogues as Synkavit is thought to be a possible cause of hyperbilirubinaemia due to increased haemolysis with risk of brain damage (* 10). In our series the total dose of vitamin K (menadiolsodiumsulphate) has never been more than 5 mg None of our patients has been treated with gantrisin (14). In all 3 of our patients who at follow up were found to have cerebral damage there were circumstances other than hyperbilirubinaemia to account for injury at the time of birth. This is not to say with certainty that hyperbilirubinaemia might not have had a contributing factor to the brain lesion. We have not found any evidence that in the absence of haemolytic disease hyperbilirubinaemia *alone* in full

term vigorous infants gives rise to cerebral lesions irrespective of whether or not exchange transfusion is performed. It would appear that the custom of performing exchange transfusion on these infants could be largely dispensed with, a change which would be of considerable practical advantage since hyperbilirubinaemia has such a high incidence. It is however not unlikely that exchange transfusion is worth while in those cases where hyperbilirubinaemia is associated with circumstances favourable to impairment of the blood brain barrier or to the liberation of bilirubin from an albumin bilirubin complex, e. for instance after gantrisin medication or with a low serum albumin concentration (13).

The incidence of infants with hyperbilirubinaemia is much higher than corresponding figures from England (16). This may possibly by explained through the higher dosage of prophylactically given vitamin K. This problem is under investigation.

Summary

One hundred and thirteen full-term infants with bilirubin values of 18 mg or more during the neonatal period, have been followed-up at the age of 2–3 years, and compared with 51 full-term, non-icteric infants, born during the same period. Exchange transfusion was carried out in 30 of the children with jaundice. No cases of kernicterus could be demonstrated. Play audiometry was carried out in 125 children (86 with jaundice, 39 controls). No deafness of central type was revealed. At the paediatric follow-up examination no relevant abnormalities were found. Merrill–Palmer testing produced evidence of a similar I.Q. distribution among the icteric as among the non-icteric children. A cerebral lesion was diagnosed in 3 cases, but in all these another aetiology (disturbance at the time of birth) occurred in addition to hyperbilirubinaemia. Hyperbilirubinaemia alone did not give rise to any cerebral lesion.

References

1. BARKER, J. C. W. Über den Icterus gravis und Kernicterus bei Frühgeburten ohne nachweislichen ABO- und Rhesus-Blutgruppenantagonismus. *Acta Paediat (Upps)*, *44* 520 1954.
2. BOUND, J. P. and TELFER, T. P. Effect of vitamin K dosage on plasma bilirubin levels in premature infants. *Lancet I* 720 1956.
3. BROWN, A. K. and ZUELZER, W. W. Studies in hyperbilirubinaemia. I. Hyperbilirubinaemia of the newborn unrelated to isoimmunization. *Amer J Dis Child*, *93*, 263, 1957.
4. CROSSE, MARY V., WALLIS, PATRICIA G. and WALSH, ANNA M. Replacement transfusion as a means of preventing kernicterus of prematurity. *Arch Dis Child* 33 403 1958.
5. DIAMOND, L. K., ALLEN, F. H. JR., VANN, D. D. and POWERS, J. B. Erythroblastosis foetalis; Round table discussion. *Pediatrics 10* 337 1952.
6. ERNSTER, L., HERLIN, L. and ZETTERSTRÖM, R.: Experimental studies on the pathogenesis of kernicterus. *Pediatrics, 20* 647 1957.
7. HSIA, D. Y. Y., ALLEN, F. H., J. GELLIS, S. S. and DIAMOND, L. K.: Erythroblastosis foetalis. VIII. Studies of serum bilirubin in relation to kernicterus. *New Engl J Med 247* 668, 1952.
8. JENDRASSIK, L. and GRÓF, P. Vereinfachte photometrische Methode zur Bestimmung des Blutbilirubins. *Biochem Z* *297* 81 1938.
9. KILLANDER, A., MULLER-EBERHARD, U. and SJÖLIN, S. Indications for exchange transfusion in newborn infants with hyperbilirubinaemia not due to haemolysation. *Acta Paediat (Upps)*, *49* 377 1960.
10. MEYER, T. C. and ANGUS, J. The effect of

large doses of "Synkavit" in the newborn. *Arch Dis Child* 31 78, 1956.
11. Mollison P. L. and Cutbush, Marie: A method of measuring the severity of series of cases of haemolytic disease of the newborn. *Blood*, 6 777 1951
12. Mores, A., Fabiánová, I. and Mesáriková, E.: The relation of hyperbilirubinaemia in newborns without isoimmunisation to kernicterus. *Acta Paediat (Upps)*, 43 [illegible], 1954.
13. Odell, G. B.: The dissociation of bilirubin from albumin and its clinical implications. *J Pediat* 55 268 1959
14. Silverman W. A., Andersen, D. H., Blanc, W. A. and Crozier, D. N.: A difference in mortality rate and incidence of kernicterus among premature infants allotted to two prophylactic antibacterial regimens. *Pediatrics*, 18 614 1956.
15. Stutsman, R.: Guide for Administering the Merrill-Palmer Scale of Mental Tests. World Book Company New York 1948.
16. Zetterström, R.: Personal communication.

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Gamma Globulin against Rubella in Pregnancy

I Prevention of Maternal Rubella by Gamma Globulin and Convalescent Gamma Globulin a Follow-Up Study

by ROLF LUNDSTRÖM CLAES THORÉN and BERNT BLOMQUIST

Introduction

Gregg s [9] discovery in 1941 of maternal rubella as a cause of congenital abnormalities has been clearly confirmed in numerous studies on the subject. Different methods of collecting data have given varying results with respect to the incidence of congenital anomalies after maternal rubella in early pregnancy Siegel & Greenberg [*6] presented some evidence that the incidence may differ depending on occurrence of rubella during epidemic or non-epidemic years. The risk is high enough [5 17 20 *4] to make any attempt to prevent the disease in early pregnancy well worth while [20 24].

Active immunization programmes with deliberate exposure of young girls have been attempted [1 11] The practical difficulties of inducing immunity in large populations along these lines limit the value of such trials. As long as the rubella virus ha not been isolated and no effective vaccine has been produced, there will always be a certain number of susceptible fertile women who should avoid contact with cases of rubella in early pregnancy This is not however always possible Consequently when such a woman has been exposed to rubella after recent conception, the only conceivable method of protecting her is by passive immunization, e.g with convalescent gamma globulin or ordinary pooled gamma globulin

Earlier Investigations

In the past, several attempts to protect pregnant women and other susceptible subject by passive immunization have been made. Table 1 summarizes a number of studies in this field, mostly showing favourable results, but with attack rates in the treated subjects ranging from 0 t 41%. Even though passive immunization seems to have had a protective effect against the development of manifest signs of maternal rubella in many instances, this is not necessarily equivalent to success in the prevention of defects, since inapparent disease may cause damage to the foetus [25]. Rubella without a rash has also been described [15].

Little is known of the results of pregnancies in which passive immunization against rubella was undertaken [2], and reports on the offspring in such cases include only observation at birth. Kamerbeek [11] for instance reported the results of giving 75-100 ml of rubella convalescent serum to age susceptible pregnant women exposed to rubella. None of the women contracted

TABLE 1 *Passive immunization against rubella.*

Reference	Method	No. of individuals	Attack rate %
Barenberg *et al.* 1943 (4)	Pooled plasma	Not stated	0
Aycock & Ingalls 1946 (3)	Pooled gamma globulin	4 children	Y protect.
Stokes 1947 (27)	Pooled gamma globulin 5 ml	5 adults	0
Kamerbeek 1949 (11)	Convalescent serum 75–100 ml	200 pregnant	0
Landon *et al.* 1946 (16)	Pooled gamma globulin 6 ml	132 children	5
	Controls	129 children	17
Korns 1952 (12)	Pooled gamma globulin 0 1 ml/lb	Children and adults	
	( ) No. 3214–2	199	13
	Controls	201	13
	(b) No. C 2768	83	0
	Controls	82	0
	( ) No. C 2763	45	20
	Controls	60	58
	(d) No. C 3227	28	29
	Controls	46	39
Anderson & McLorinan 1953 (2)	Convalescent gamma globulin		
	( ) 2 ml	424 pregnant	1
	(b) 4 ml	358 pregnant	1
	( ) 4 ml	45 boys	7
	Controls	46 boys	15
	(d) Experimentally induced rubella 4 ml	18 adults	83
	Controls	9 adults	33
Lundström 1952 (18)	Convalescent serum 25–50 ml	98 pregnant	(?)
Ward & Parker 1956 (28)	Convalescent serum 30 ml	541 pregnant	0.9
	Controls	102 pregnant	10.8
	Controls	50 not pregnant	12.0
Krugman & Ward 1958 (14)	Experimental infection		
	Normal serum (no antibody)	6 children	83
	Pooled gamma globulin	6 children	17
	Convalescent plasma	6 children	17
	Convalescent gamma globulin	6 children	0
Hoster & Schaler 1958 (9)	Pooled gamma globulin 15 ml	200 soldiers	0
	Pooled gamma globulin 5 ml	200 soldiers	0.
	Placebo	200 soldiers	2.3
Grayston & W tten 1959 (7)	Pooled gamma globulin 5 ml	197 boys	8.6
	Convalescent gamma globulin 2 ml	198 boys	10.2
	Placebo controls	203 boys	14.1
	Uninoculated controls	194 boys	20.4
Present investigation	Convalescent gamma globulin 4 ml	251 pregnant	2.4
	Gamma globulin 24 ml	28 pregnant	0

rubella, 15 (7.5%) had spontaneous abortions, and three infants (1.5%) showed anomalies presumably not belonging to the rubella syndrome. A total of 180 infants appeared healthy at birth, and three pregnancies were not completed at the time of writing.

TABLE 2. *Results of pregnancy in 98 women given 25–50 ml of convalescent serum after exposure to rubella (18) follow-up examination of children at 1–3 years of age The number of offspring is 100 since two pairs of twins are included*

Result of pregnancy	No. of children following treatment of mothers exposed to rubella in month of pregnancy						Comments
	I	II	III	IV	>V	Total	
Spontaneous abortion			3			5	Full-term, cause unknown.
Stillbirth		1	1			2	Maternal toxaemia.
Neonatal death		2[3]	1		2[4]	5	Premature pairs of twins following maternal toxaemia.
Congenital heart disease			1			1	Birth injury
Normal at birth, no follow-up				1	4	5	Probably Fallot's tetralogy
Normal at follow-up	1	21	19[7]	21	15	82	One case of funnel-chest, otherwise normal.
Total	1	26	23	22	21	100	

Treatment with Convalescent Serum

One of us (R.L.) collected a series of 98 pregnant women, with no previous history of rubella and exposed to the disease during an epidemic in Sweden in 1951 [17] and treated with 25–50 ml of rubella convalescent serum [18, 19]. Two women reported a rash some days after treatment, apparently not characteristic of rubella; they were not seen by a physician. Both had normal children. The results of follow-up examinations of the children of prophylactically treated women up to the age of 1 to 3 years are seen in Table 2. Five pregnancies ended in spontaneous abortion and five in perinatal death, four due to obstetrical complications, the fifth being a stillbirth at term (cause unknown). One child was funnel-chested, at follow-up normal after surgery One child whose mother was pregnant in the third month when treated for exposure to rubella was reported normal at birth but was found at follow-up to be a blue baby probably due to Fallot's tetralogy Five children were not reexamined after birth, one was exposed in the fourth month, the others later in pregnancy Reexamination of the remaining children did not reveal any abnormalities.

This series, which contains one instance of congenital heart disease as the only anomaly that could be ascribed to the action of inapparent rubella despite passive protection, is too small to be conclusive The incidence of 2% of (questionable) rubella after treatment is low Previous reports on rubella prophylaxis in pregnancy have not included systematic follow-up studies of the children of the women treated. Consequently further information on the value of passive immunization against rubella in pregnancy is highly desirable.

An epidemic of rubella in Stockholm in 1955–1956 gave an opportunity for further studies of the subject

Case Material

In 1956, the health authorities of the City of Stockholm resolved that susceptible pregnant women were to be given, free of charge, passive immunization against rubella if they were less than 4 months pregnant and were referred to Kronprinsessan Lovisas Barnsjukhus by the maternity welfare centres, or by private physicians, on account of exposure to rubella. Altogether 65 of the 29 women referred were not treated, for one of the following reasons: exposure had been unlikely; more than 2 weeks had lapsed since exposure; they had been pregnant for more than 4 months; they had an earlier

history of rubella. Immunization was thus given to 327 women. When collecting the series, 48 women were excluded, since—according to the stipulations—they should not have been treated. Thus, 279 definitely exposed women remained for the study 251 of them were treated with 4 ml of convalescent gamma globulin, and 28 with 4 ml of pooled gamma globulin, since the former was not available during a short period.

The purpose of this study was to examine the children of all the women treated. As five women had emigrated and three could not be traced at the registered address, 271 of the 279 (97 1 %) were included in the fol low up.

Altogether 38 % of the women were housewives without other occupation, 27 % were office personnel, 16 % worked in hospitals or physicians offices, 12 % were teachers, and 8 % had some other form of work. Seven per cent were less than 20 years, 60 % between 21 and 30 years and 33 % more than 30 years old.

During the epidemic, number of preg nant women who had contracted rubella were treated with convalescent serum gamma globulin. The outcome of their pregnancies will be reported in a subsequent paper [21].

Methods

The convalescent serum gamma globulin used in this study consisted of gamma globulin, prepared from the blood of donors who had had an attack of rubella within the previous 4 months. The blood was collected by the National Bacteriological Laboratory Stockholm, mainly through co-operation with the medical fficers of the armed forces. The serum was fractionated according to a modification of Cohn method 10 by AB KABI Stockholm. The pooled gamma globu lin consisted f similarly produced gamma globulin (KABI) from retroplacental blood collected in maternity hospitals. The dosage was 4 ml f convalescent gamma globulin intramuscularly; when pooled gamma globulin was used, 4 ml were injected by the same route in both cases the solution was 12 %.

After treatment, the women were given a form to be sent to the investigators, report ing rash if occurring, outcome of pregnancy name of delivery hospital, and present stat of baby s health.

When the children were 2 to 3 years ld, they were summoned by us for examination at Kronprinsessan Lovisas Barnsjukhus. Twenty children were examined at other children hospitals and child welfare centres, or by private practitioners, mainly paediatricians.

Examination of the children was thorough, with special attention t the eyes, ears, heart, and mental development; in the cases examined by the present authors, it included ophthalmoscopy Children with strabismus and/or other visual disturbances were referred for special ophthalmologic examination. When a heart murmur was present, the ECG and PCG were recorded, and the heart X-rayed. Children suspected of impaired hearing were examined by play audiometry at the Laboratory of Audiology Karolinska Sjukhuset. When mental retardation was suspected, Terman–Merrill tests were made. The case records of children with complications at delivery and f those admitted to hospital were studied, as were the records of cases reported as abortions.

Results of Treatment with Convalescent Gamma Globulin

Six of the 251 women contracted rubella despit treatment within 2 weeks of ex posure (Table 3). In three of these cases pregnancy resulted in abortion, and one woman gave birth to a child with signs of the rubella syndrome

Case 1 A 25-year-old Para II. Last men struation Jan. 19 1956. She was exposed to rubella on March 6, and was given 4 ml of convalescent gamma globulin on March 10. Rubella ppeared on March 24 she was given 12 ml f convalescent gamma globulin on the same day On Oct. 27 she was delivered f a boy weighing 2660 g, and without obvious

TABLE 3 *Six cases of maternal rubella after treatment with 4 ml of convalescent gamma globulin, i.e. 2.4 per cent of total 251 women treated.*

Result of pregnancy	Rubella in month of pregnancy I	II	III	IV	Comments
Spontaneous abortion		1	2*		
Rubella syndrome			1		Case 1 see text.
Immature, normal at follow-up			1		
Full term, normal at follow-up			1		

* One woman also given 12 ml of convalescent gamma globulin after onset of rubella.

defects. On examination at 3 years of age he was found to show slight mental retardation, hearing impairment, and signs of chorioretinitis.

The other two women who, after treatment, contracted rubella in the third month of pregnancy gave birth to children who were healthy at examination when they were 2-4 years old.

Of the remaining 245 women who did not contract manifest signs of rubella after treatment 16 (6.5%) aborted (Table 4).

Severe malformations were present in three children i.e. congenital cataract patent ductus arteriosus and mitral stenosis in one case (reported in detail by Malmberg *et al* (23)) myelomeningocele with internal hydrocephalus in one and agenesis of the corpus callosum in one case. One case of moderate mental retardation (IQ 70) one of a dermoid cyst and two cases of hypertrophic pyloric stenosis were also recorded.

Among the other children slight strabismus was recorded in 3 cases without other abnormalities. Minor defects, such as haemangioma of the skin testicular hydrocele and inguinal hernia, were considered as unimportant and were therefore not classified as abnormalities.

Results of Treatment with Pooled Gamma Globulin

Altogether 28 women were treated with 24 ml of pooled gamma globulin (KABI) after exposure to rubella (Table 5). All escaped an attack of the disease. Two women aborted. Two children were not available for examination. One case of mongolism was observed.

Case 2. A 25-year-old Para III. Last menstruation April 25 1956. She was exposed to rubella on June 20, and treated with 24 ml of pooled gamma globulin on July 3. Pregnancy was uneventful. On Feb. 7 1957 she was delivered of a girl with mongolism. The child was treated in a children's hospital for pharyngitis and mongolism in July 1958, when no other anomalies were detected.

Discussion

Since rubella is a clinical entity due with great certainty to a virus infection, protection by passive immunization with

TABLE 4. *Results of pregnancy (248 children) in 245 women given 4 ml of convalescent gamma globulin after exposure to rubella in first 4 months of pregnancy none contracted rubella Repeated exposure and treatment in 12 cases only 1st exposure listed in table (Another 6 women contracted rubella despite treatment see Table 3)*

Result of pregnancy	No. of children following treatment of mothers exposed to rubella in month of pregnancy				
	I	II	III	IV	Total
Spontaneous abortion	—	12	3	1	16
Stillbirth	—	1	—	2[1–3]	4
Death	—	1	3[4–6]	3[7–]	7
Surviving children					
Congenital heart disease	—	—	—	1	1
Cataract	—	—	1[13]	—	1
Other abnormalities	—	2[14, 15]	2[16]	1[17]	5
Birth injuries with sequels	—	—	1	1	2
Normal at birth without follow-up	—	2	3	—	5
Birth-weight < 2 500 g, normal at follow-up	—	4	2	1	7
Full-term, normal at follow-up	3	66	71	62	194
Three pairs of twins	—	—	4[18, 19]	2[20]	6
Total	5	78	90	75	248

Macerated foetus.
[2–3] Birth injuries.
Intrauterine asphyxia.
Toxoplasmosis.
Cataract and VSD
Meningomyelocele.
Agenesis of corpus callosum.
Capillary bronchiolitis.
[10–11] Birth injuries.
[12] Minute ventricular septal defect, Roger type
[13] Small unilateral polar cataract, normal vision.
[14] Dermoid cyst.
[15] Mental retardation: IQ 79.
[16] Hypertrophic pyloric stenosis.
[17] Urinary tract anomaly
[18] Both twins normal at follow-up.
[19] One twin stillborn macerated, other normal at follow-up.
[20] One twin died of pulmonary atelectasis; other normal at birth, had emigrated at follow-up.

serum or gamma globulin should be theoretically possible. Past experience with varying amounts of convalescent serum and gamma globulin has not given uniform results, as can be seen in Table 1 This may be due to differences in preparation and/or dosage and/or in the amount of circulating antibodies in the donors. The antibody content cannot be determined in the laboratory Consequently clinical observations—with their inherent inaccuracy—are the only means hitherto available for evaluating the efficacy of immunizing procedures against rubella.

In the present series, it was an advantage that the women were referred to a single children's hospital. This allowed control of the indications for treatment and resulted in only a relatively small

TABLE 5 *Results of pregnancy in 28 women given 21 ml of pooled gamma globulin after exposure to rubella None contracted rubella*

Result of pregnancy	No. of children following treatment of mothers exposed to rubella i month of pregnancy					Comments
	I	II	III	IV	Total	
Spontaneous abortion		1	1			
Abnormalities			1		1	One case of mongolism
Normal at birth, not examined later		1		1	2	Two had emigrated
Normal at birth and at follow-up		9	9	5	23	
Total		11	11	6	28	

number of cases being lost for the follow up study

That the epidemic in question had teratogenic effects has been shown by abnormalities of the rubella type in children observed by us [21] and the cases of rubella deafness reported by Barr & Lundström [5]

Since we could not obtain representative controls—in view of the premises for this survey—the possibility of judging the protective effects by the incidence of manifest rubella after treatment was limited in our series. The incidence of rubella in definitely exposed, susceptible pregnant women was 2 4 per cent (6 of 251). In our opinion, this figure is lower than could have been expected without treatment The number of exposed women given 14 ml of ordinary pooled gamma globulin is too small to permit any conclusions. It may however be recalled that none of these 28 women contracted rubella.

The possibility of attenuating rubella by administration of gamma globulin must be considered since it is known to have this effect in measles Inapparent rubella may produce anomalies of the same kind as the manifest disease. The relatively high incidence of spontaneous abortions, 12 of 78 (15 %) when exposure occurred in the second month of pregnancy might support this assumption, even though the overall incidence covering the first four months of pregnancy was only 8.5 %. However only two out of 245 children (0.8 %) whose mothers were exposed to rubella and after treatment with convalescent gamma globulin, apparently did not contract the disease showed abnormalities belonging to the rubella syndrome One was in the form of both cataract and congenital heart disease and the other had a presumably unimportant ventricular septal defect Compared with the 9% incidence of eye, ear and/or heart defects in a similarly studied material of rubella in early pregnancy in 1951–52 [22], this figure is low and corresponds far better with the 1 6 % of similar anomalies found in the control group of the same study The occurrence of two gross malformations of the central nervous system is noteworthy but inconclusive as is one case of mental retardation. The small polar cataract in one case was not of the type seen after maternal rubella.

It is, however known that defective hearing due to maternal rubella is not infrequent (approximately ?0 %) when slight hearing loss is included [5] The incidence of the latter can be estimated only by using audiometric examination in all cases. Since we did not have such facilities, cases of slight hearing loss may not have been recognized in our series.

At the time when this investigation was started, the recommended dose of convalescent gamma globulin for protection of pregnant women was 4 ml [2]. It is possible that a larger dose could have prevented occurrence of the malformations described. Krugman & Ward [14] recommended 10 ml of convalescent gamma globulin or 20 ml of pooled gamma globulin (16 %) for the prevention of rubella in early pregnancy Controlled series with this dosage are not known by us. Even though the dosage used in the present study seemed to afford protection our results have given reason to advise the higher dosage and at present 1 ml of (12 %) convalescent gamma globulin are administered for prevention of rubella in susceptible pregnant women in Sweden [6]. If such gamma globulin is not available administration of 24 ml of pooled gamma globulin is recommended for this purpose.

Since it was impossible to obtain controls, no definite proof can be given of the efficacy of passive immunization in our series The relatively low incidence of rubella in adequately treated women, as well as the extremely few observations of defects possibly due to maternal rubella, are nevertheless encouraging Consequently it is recommended that passive immunization of susceptible women, when exposed to the disease in the first four months of pregnancy be continued. Additional follow-up studies of the results with respect not only to the incidence of rubella after treatment but also to the resulting children up to the age of about 3 years, are therefore desirable

Summary

In the present investigation, 251 women —who in 1956 had been exposed to rubella in the first 4 months of pregnancy—were given 4 ml of convalescent gamma globulin. An account is given of a follow up study with examination of their children at 2–3 years of age. All but 5 cases were traced. Despite treatment six women contracted rubella, i.e an attack rate of 2 4 %. Three of these six women aborted, and one had a child with malformations of the rubella syndrome type

Two of the _45 women showing no signs of manifest rubella had children with defects that might be ascribed to inapparent maternal rubella

Another 28 pregnant women were given _4 ml of ordinary pooled gamma globulin. None contracted rubella, two had spontaneous abortions, and one gave birth to a child with mongolism.

The present investigation indicates that convalescent gamma globulin and ordinary gamma globulin have a protective effect against rubella.

The following dosage is recommended for future use in pregnant women exposed to rubella. Rubella convalescent gamma globulin (1_ %) 12 ml i.m. as soon as possible after exposure when this is not available pooled gamma globulin (1_ %) _4 ml i.m.

References

1 Andersson S. G Epidemiological aspects of rubella. *Med J Aust, II* 389, 1950.

2. Andersson, S. G and McLorinan H.: Convalescent gamma globulin as prophylactic against rubella. *Med J Aust I* 182, 1953.

3. Aycock, W L. and Ingalls, T H. Maternal disease as a principle in the epidemiology of congenital anomalies. *Amer J Med Sci, 212* 366, 1946.

4. Barenberg L. H., Levy W Greenstein N M. and Greenberg, B Prophylactic use of human serum against contagion in a pediatric ward. *Amer J Dis Child 63* 1101, 1942.

5. Barr, B. and Lundström, R.: Deafness following maternal rubella. Retrospective and prospective studies. *Acta Otolaryng (Stockh) 53* 413, 1961

6. Blomquist B Lundström, R. and Thorén C.: Passive immunization against rubella in pregnancy A follow-up study (Proc. Ped. Soc.) *Acta Paediat (Uppsala), 49* 653, 1960.

7 Grayston J T and Watten R. H.: Epidemic rubella in Taiwan 1957–58. III. Gamma globulin in the prevention of rubella. *New Engl J Med 261* 1145, 1959.

8. Gregg, N M. Congenital cataract following German measles in the mother *Trans Ophthal Soc Aust 3* 35, 1941.

9 Houser, H. G. and Schalet, N Prevention of rubella with gamma globulin. *Clin Research, 6* 281 1958.

10. Ingalls, T H., Babbot F L., Hampson K. W and Gordon J E.: Rubella. Its epidemiology and teratology *Amer J Med Sci, 239* 363, 1960.

11. Kamerbeek, A. E. H. M.: Het rubella probleem in het licht van nederlandse ervaringen. *Verh Inst Praevent Geneesk* XIV Stenfert Kroese Leiden, 1949

12. Korns, R. F Prophylaxis of German measles with immune serum globulin. *J Infect Dis, 90* 183, 1952.

13. Krugman S. and Ward R.: The rubella problem. Clinical aspects, risk of fetal abnormality and methods of prevention. *J Pediat 44* 489 1954.

14. Krugman S. and Ward R. Rubella. Demonstration of neutralizing antibody in gamma globulin and re-evaluation of the rubella problem. *New Engl J Med, 259* 16 1958.

15. Krugman S., Ward R., Jacobs, K. G. and Lazar, M. Studies on rubella immunization. I. Demonstration of rubella without rash. *JAMA, 151* 285, 1953.

16. Landon J F., Bass, M., Dwyer H. B., Foote, F and Muckenfuss, R.: Efficacy of gamma globulin in prevention of German measles. *New York J Med, 49* 21 1949.

17 Lundström, R.: Rubella during pregnancy Its effect upon perinatal mortality the incidence of congenital abnormalities and immaturity *Acta Paediat (Uppsala), 51* 583, 195..

18. — Passive immunization against rubella during pregnancy Atti del VI Congresso Internazionale di Microbiologia, Roma, 6–12 Settembre 1953. Vol. 3, Sez. VIII Pag 88.

19 — Gammaglobulin in Prophylaxe und Therapie bei Infektionskrankheiten. *Neue Oest Z Kinderheilk, 1:* 193, 1955/56.

20. Lundström R. and Boström, I. L.: Rubella in pregnancy and the incidence of developmental anomalies of the eyes. *Acta Ophthal (Kbh), 30* 782, 1952.

21. Lundström, R., Thorén C. and Blomquist B Gamma globulin against rubella in pregnancy II. Maternal rubella treated with convalescent gamma globulin. A follow-up study *Acta Paediat (Uppsala), 50* 453, 1961.

22. Lundström, R.: To be published.

23. Malmberg, N. Sterner, G. and Lundmark, C.: Asymptomatic maternal rubella as a probable cause of embryopathy *Ann Paediat Fenn, 3* 437 1957

24. Manson M. M., Logan W P D. and Loy R. M Rubella and other virus infections during pregnancy Reports on Public Health and Medical Subjects No. 101 Ministry of Health, Her Majesty's Stationery Office, London, 1960.

25. Schick, B.: Diaplacental infection of the fetus with the virus of German measles despite immunity of the mother *Acta Paediat (Uppsala), 43* 563, 1954.

26. Siegel, M. and Greenberg, M.: Fetal death, malformation and prematurity after maternal rubella. Results of prospective study 1949–1958. *New Engl J Med, 262* 389, 1960.

27 Stokes, J J The use of gamma globulin from large pools of adult blood plasma in certain infectious diseases. *Ann Intern Med, 26* 353, 1947

28. Ward, H. and Parker, G.: Passive protection against rubella. *Med J Aust, I* 81 1956.

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Gamma Globulin against Rubella in Pregnancy

II Manifest Maternal Rubella in Early Pregnancy Treated with Convalescent Gamma Globulin a Follow-Up Study

by ROLF LUNDSTRÖM, CLAES THORÉN and BERNT BLOMQUIST

The controversial estimates of the risk of congenital abnormalities have led to different opinions on the management of a woman with rubella in early pregnancy Some authors have recommended therapeutic abortion [4], whereas others are strictly opposed to an intervention of this type from religious, medicolegal and/or medical points of view The matter has recently been discussed by Jeffcoate [6], who found an interruption of pregnancy in such cases acceptable only when a maternal psychiatric indication existed. In any event, induction of abortion is a poor solution of the problem.

Little is known of the possibility of protecting the foetus of a mother with already manifest, early maternal rubella from congenital defects by means of passive immunization. During an epidemic of rubella in Sweden in 1951 a few cases of rubella in pregnancy were treated with 25–50 ml of convalescent serum 1–21 days after appearance of the rash [8]. Three women were pregnant in the third month four in the fourth month and two were in later stages of gestation. All nine had full-term infants normal at birth At examination at 1–3 years of age, eight were completely healthy the remaining child could not be traced However in view of the small number of observations, the results were inconclusive By chance unilateral perceptive deafness was detected in one child—whose mother had been given convalescent serum for rubella in the fourth month of pregnancy—at the age of 5 when the investigation was finished.

Another epidemic of rubella occurred in 1956. Since a number of women with manifest maternal rubella were treated with convalescent gamma globulin we had an opportunity of making a prospective study of their offspring The results of prophylactic treatment of pregnant women exposed to rubella have been described separately [5, 10].

Case Material and Methods

In 1956, every physician in Sweden who so wished was given, free of charge, 12 ml of convalescent gamma globulin for administration—as soon as possible after appearance of the rash—to each case of rubella in the first 4 months of pregnancy This dose was three times as large as the 4 ml dose chosen for prophylaxis. Totally 28 women were treated in this way The average interval

TABLE 1 *Results of pregnancy in 27 cases of maternal rubella given 12 ml of convalescent serum on the average 2.6 days after appearance of the rash*

Result of pregnancy	No. of cases	Rubella in month of pregnancy I	II	III	IV	Comments
Induced abortion	2		1	1		
Spontaneous abortion	6	1	2	3		One case of missed abortion.
Stillbirth	1			1		Erythroblastosis (Rh-immunization).
Neonatal death	1			1		Immaturity birth weight 1450 g.
Rubella syndrome	4			4		
Other abnormalities	1				1	Funnel-chest
Observed at birth only	2			1	1	One normal, one with club-foot.
Normal at 3–4 years	9		1	4	4	
Twin birth	1		1			One twin died at birth, one normal at follow-up.
Total	27	1	5	15	6	

between appearance of rash and treatment was 2.6 days (range 0–7 days). Three of the women also belong to Part I of this study [10], since they had been treated prophylactically shortly after exposure as well. Reports on the outcome of pregnancy and the condition of the children at 3–4 years of age were collected as described in Part I [10]; all but five children were examined by us. Data were available in 27 cases; the remaining woman could not be traced. Two children were observed at birth only; at the follow up examination, they were living abroad.

Results

The results of the study are seen in Table 1 Abortion was induced in 3 of the 27 cases studied. One of them was, however disclosed as a missed abortion. Five women miscarried. If the induced, missed abortion is included, 6 of 25 pregnancies (24 %) terminated in early foetal death. In the 19 pregnancies at risk in the first trimester the abortion rate was 32 %. Immaturity at birth was observed in one pair of twins, one of whom died shortly after birth the other being healthy at follow up Another single immature died on the first day Autopsy disclosed no anatomic malformations in these cases. Four children with maternal rubella in the third month showed anomalies usually ascribed to maternal rubella, i.e. chorioretinitis and perceptive hearing loss in two cases, chorioretinitis alone in one, and in one case multiple abnormalities including bilateral cataract congenital bilateral glaucoma, mental retardation, hypospadias and pseudohermaphroditism One child had a funnel-chest, but was otherwise normal.

When, on one occasion, convalescent gamma globulin was not available a mother pregnant in the second month was given 40 ml of pooled gamma globulin on the day of appearance of the rubella rash. The resulting infant was a full term boy weighing 3030 g, reported normal at birth. At follow-up, feeding difficulties were stated to be present. At 3 years old he showed signs of malnutrition, and had alternating strabismus. Special investigations disclosed no other eye anomalies, nor defects of the ears, heart, or mental development.

Discussion

When certain virus diseases, e.g. measles, smallpox and chickenpox, occur in a pregnant woman shortly before term, the infant may contract the disease in question after birth, the interval between onset in mother and in child corresponding to the expected incubation period. It is uncertain whether there is an incubation period in early pregnancy as well. Presuming that it does exist the teratogenic effect might be mitigated by giving antibodies in large quantities as soon as possible after the diagnosis of maternal rubella. Measurable amounts of certain antibodies are in fact, known to pass the placental barrier as early as in the middle of gestation [14].

Only a few observations on the results of treatment of maternal rubella by passive immunization have been published. Bass [7] reported a pregnant woman who was given gamma globulin after she had contracted rubella, the resulting infant had defects. Berge [3] gave an account of six women with rubella, treated with 75–100 ml of convalescent serum. Spontaneous abortion occurred in two cases one gave birth to a defective infant and three had normal infants In the *Journal of the American Medical Association* 1956 [15] the advice for a pregnant woman with rubella three months and five days pregnant was: Gamma globulin should be given to the patient, because it will do her no harm and it may be helpful to the fetus. Spoto [13] recommends passive immunization to 11 cases of maternal rubella in early pregnancy Thus believers in passive immunization in this situation do in fact exist

The outcome of the investigation has shown that, under the circumstances in question the benefit was apparently none. The high incidence of early foetal death as well as of abnormalities in the survivors, with great probability due to rubella, are comparable with recent findings of the incidence of abnormalities in prospective studies of maternal rubella not treated by passive immunization [1 9 11 1.]

The quantity of convalescent gamma globulin used for treatment of the cases in the present series was three times as large as that used for the apparently successful prophylaxis. Despite this, the unsuccessful outcome of the present trial might be explained by an insufficiently high dose of convalescent gamma globulin. Another possible explanation is that the mechanism of transfer of virus from mother to foetus is not influenced by passive immunization, since passage has already occurred before appearance of the rash in the mother We cannot count on a neutralization of virus already transmitted to the foetus, since in these early stages no antibody transfer seems to take place

Judging by our series, administration of 1 ml of 12 % convalescent gamma globulin does not seem to prevent foetal damage in cases of early maternal rubella It is conceivable that much larger doses would have been effective We do not, however believe that there is much likelihood of passive immunization after appearance of the disease being successful in preventing such damage Consequently the National Bacteriological Laboratory has abandoned the recommendation of convalescent gamma globulin for cases of manifest maternal rubella

Present resources do not allow a renewed trial with larger amounts of antibodies which, in such case, should be given immediately on onset of the disease

Rubella virus cannot be propagated for the production of a vaccine. Active immunization by deliberate exposure of young girls to rubella should therefore be encouraged as far as possible. If active immunity has not been acquired by a woman in early pregnancy exposed to rubella, convalescent gamma globulin or pooled gamma globulin should be administered as soon as possible after exposure

Summary

Rubella convalescent gamma globulin was given to 28 women with rubella in early pregnancy Of those treated in the first trimester pregnancy terminated in foetal death in 3. % and 11 of 21 offspring survived. At the age of 3-4 years, four of the 21 survivors showed abnormalities belonging to the rubella syndrome. Administration of antibodies to women with manifest rubella in early pregnancy did not succeed in preventing foetal damage

References

1 BARR, B and LUNDSTRÖM, R.: Deafness following maternal rubella. Retrospective and prospective studies. *Acta Otolaryng (Stockh)* *53* 413, 1961
2. BASS, M. H.: Recent advances in our knowledge of the contagious diseases of childhood. *Bull N Y Acad Med*, *24* 784 1948.
3. BERGE, B. S. cit. KAMERBEEK (7).
4 BELL, J On rubella in pregnancy *Brit Med J* *1* 686, 1959.
5. BLOMQUIST B LUNDSTRÖM, R. and THORÉN C. Passive immunization against rubella in pregnancy A follow-up study (Proc. Ped. Soc) *Acta Paediat (Uppsala)*, *49* 632, 1960.
6. JEFFCOATE, T N A.: Indications for therapeutic abortion. *Brit Med J* *1* 581 1960.
7 KAMERBEEK A. E. H. M.: Het rubella probleem in het licht van nederlandse ervaringen. *Verh Inst Praevent Geneesk* XIV Stenfert Kroese, Leiden, 1949
8. LUNDSTRÖM, R Gammaglobulin in Prophylaxe und Therapie bei Infektionskrankheiten. *Verh Oest Z Kinderheilk* *1* 183, 1955/56.
9 LUNDSTRÖM R. and BOSTRÖM, L. L.: Rubella in pregnancy and the incidence of developmental anomalies of the eyes. *Acta Ophthal (Kbh)*, *36* 782, 1958.
10. LUNDSTRÖM, R., THORÉN C. and BLOMQUIST B.: Gamma globulin against rubella in pregnancy I. Prevention of maternal rubella by gamma globulin and convalescent gamma globulin. A follow-up study *Acta Paediat (Uppsala)*, *50* 444, 1961.
11 LUNDSTRÖM, R.: T be published.
12. MANSON M. M., LOGAN W P D and LOY R. M. Rubella and other virus infections during pregnancy Ministry of Health Reports on Public Health and Medical Subjects No. 101 Her Majesty Stationery Office, London, 1960.
13. SPOTO, P.: Embriopatie da malattie infettive in gravidanza. *Minerva Ginec*, *2* 217, 1950.
14. VAHLQUIST B The transfer of antibodies from mother t offspring. *Advanc Pediat*, Vol. X. Yearbook Publishers, Chicago, 1958.
15. QUERIES and MINOR NOTES: Rubella and pregnancy *JAMA* *162* 456, 1956.

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Acute Respiratory Illness and Gastroenteritis in Association with Adenovirus Type 7 Infections[1]

by GÖRAN STERNER, PER GERZÉN MARIANNE OHLSON and GUNVOR SVARTZ-MALMBERG

Since 1053 adenoviruses types 1 2 3 5, and 7 [9 13] have been isolated in Sweden. In children adenovirus type 7 can apparently produce acute infections of the upper respiratory tract and in infants atypical pneumonia has been attributed to this virus [4 9 11 13, 16] No epidemic of type 7 infections has previously been reported in Sweden. However over the period July–August, 1959 a large number of adenovirus type 7 infections occurred among the population of Nacka a suburb of Stockholm [8]. In the autumn of the same year such infection was diagnosed in high frequency among the children admitted to two hospitals in Stockholm.

Materials and Methods

Over the period Aug 1–Dec 31 1959 717 children (0 t 16 years old) were admitted for acute respiratory infection to the Hospital for Infectious Diseases and the Children Hospital Samariten. Specimens for virological examination were obtained from 234 f these children. The present investigation was started because an unusually large number of children exhibiting both respiratory and gastrointestinal symptoms were hospitalized. This syndrome has earlier been attributed to adenovirus infections [10]. At the Hospital for Infectious Diseases, specimens for virological examination were obtained from most f the patients with this syndrome but only from a few of those admitted for merely acute respiratory infection. At the Children Hospital Samariten specimens were taken whether adenovirus infection was suspected or not. Specimens were, however not obtained from all patients with respiratory symptoms, mainly on account of difficulties in collecting blood specimens (e.g. in infants and in sensitive children).

In addition, 19 children (aged 4 months to 11 years) who contracted acut respiratory illness during their stay in the hospital, were examined, as were all the members f three families in which a child had been found to have infection with adenovirus type (Tables 3 and 4)

To extend the study to a control group specimens from children who had been admitted for scarlet fever were collected from 33 cases.

Water samples were obtained through the courtesy of the Stockholm Municipal Board of Health. These included raw water from Norsborg water works, which supply the

[1] The investigation was supported by grants from the Stockholm Municipal Board of Health and from Nils Malmberg' Foundation, which are gratefully acknowledged.

TABLE 1 *Virological data in 234 children admitted with acute respiratory illness (Aug.–Dec. 1959)*

Virus strains isolated from stools	Number of cases	Complement fixation test against adenovirus
Adenovirus type		
1	1	0/1
	7	2/5
3	1	0/0
5	2	0/1
6	1	0/0
7	69	61/62
Coxsackie virus type		
B3	5	0/2
B4	3	1/1
Unidentified cytopathic agents	3	0/2
No virus recovered	110	3/74
No stool specimen obtained	12	6/12
Total	234	73/180

Numerator: number of cases with significant rise. Denominator: number of cases tested.

southern parts of Stockholm and water from an open-air swimming-bath in Lake Malaren (Malarhöjdabadet) in the same area, as well as sewage from apartment houses in the south western parts of Stockholm, where most of the patients lived.

Virological methods. Specimens. Stool specimens for virus isolation were collected from 222 patients as soon as possible after their admission to hospital. From 180 of these patients two or three serum specimens were also taken, the first one on admission and the second one 8–14 days later. In 12 cases paired sera only were obtained (Table 1).

When a nosocomial infection with adenovirus was suspected, stool specimens were collected as soon as possible after the onset of illness, and serum specimens were taken in the acute phase and during the convalescent stage.

As regards the three investigated families, stool specimens and acute-phase sera were collected on the same day from all the members of each family. Convalescent sera were drawn 14 days later.

In the control group specimens of stools and blood were taken on admission and a second blood sample during convalescence.

Tissue cultures. Roller tube cultures of HeLa cells and of monkey kidney cells were used. They were prepared and maintained as previously described [18].

Virus isolation. Stool specimens. A 10% suspension was prepared in bovine amniotic fluid to which antibiotics had been added. After low-speed centrifugation 0.1 ml of the supernatant was inoculated into each of three HeLa cell cultures and 0.2 ml into each of three monkey kidney cell cultures. The medium of the HeLa cell cultures was changed after 24 hours incubation at 35°C and in all cultures after one week. If the cells remained unaffected for 14 days, the isolation test was considered negative. If cytopathic changes occurred in the HeLa cells, the culture fluid was tested for complement-fixation with a pool of human sera from convalescents after adenovirus infection. Materials negative in this test were pooled with fluids from degenerated monkey kidney cultures and passed in HeLa and monkey kidney cell cultures. The fluids from degenerated cultures were again tested for adenovirus antigen.

Water samples. One part of phosphate buffered saline of a concentration ten times the physiological one was added to nine parts of the water sample to be tested. The samples were inoculated into HeLa cell cultures, of sewage 1 ml per tube and of other samples 5 ml per tube. The medium was changed 3 hours after inoculation.

Typing of isolated virus strains. Adenoviruses were typed by neutralization test with rabbit hyperimmune sera using the technique devised by Kjellén et al. [9]. Delay of cytopathic changes for at least one week was the criterion for type identification. Isolated strains were tested against sera of types 1–17 with the exclusion of types 8 and 12. Rabbit hyperimmune sera were prepared by the method of Kjellén

et al. [9] or by technique using adjuvant immunization [15]. The sera were titrated against a large dose of homologous virus (tissue culture fluid diluted 1 10) A serum dilution that neutralized cytopathic effects for at least 10 days was employed for typing. This dilution, usually 1 2–1 10 did not neutralize other adenovirus types for longer than 4 days at most To confirm the results new material of the prototype strains 7 'Gomen and 7A 'S-1058 was obtained from the American Type Culture Collection and a rabbit hyperimmune serum against the Gomen strain from Microbiological Associates, Inc., Washington, D.C.

Complement-fixation (CF) tests The microtechnique used and the preparation of antigen have been described elsewhere [6, 9 14]. Titres are recorded as the inverted value of the serum dilution. A fourfold or greater rise in titre is considered significant.

Test for neutralizing antibodies. Patient sera were tested by the method of Kjellén et al. [9] against type 7 strain (24592/59) which had been isolated during the epidemic. A titre rise was recorded if convalescent serum inhibited the cytopathic effect for at least 3 days more than did acute-phase serum.

Bacteriological methods. From 689 children nasopharyngeal, nose and throat swabs for bacteriological examination were taken on admission.

In patients with symptoms of gastroenteritis, stool specimens were cultured for Salmonella and Shigella, in most infants also for enteropathogenic *E coli* Usually several specimens were cultured from each patient

Results

Virological results The virological data on the 234 children with acute respiratory illness are summarized in Table 1 Adenovirus strains were isolated from the stools of 101 out of 222 cases investigated for virus excretion. Eighty nine of these strains were identified as type 7 the others belonged to types 1 2, 3 5 and 6 In addition eight Coxsackie B strains[1] and three agents not yet identified were recovered.

Two of the latter three agents probably belong to the adenovirus group. Thus fluids from the degenerated H La cultures of the isolation test contained an antigen capable of fixing complement in the presence of a pool of human sera from convalescents after adenovirus infection. When tested against paired sera, the antigen gave similar titre rises as the standard adenovirus antigen employed. On passage no or only a weak cytopathic effect was observed, although the tissue culture fluid again gave a positive CF reaction. For one specimen (B 8560/59) this CF activity could only be carried through two subpassages. All passages from the other specimen (24642/59) showed CF activity and the cytopathogenicity slowly increased. Only after 10 passages was it pronounced enough for typing tests to be carried out. The strain could, however not be referred to any of types 1–7 9–11 or 13–17 It should be mentioned, that a second stool specimen obtained from the same patient 19 days later than 24642/59 was shown to contain adenovirus type 1

Paired sera were obtained from 82 of the 89 patients excreting adenovirus type 7 Sixty-one of them showed a rise in CF titre against adenovirus antigen. Of the other 21 children all but two had a titre of at least 4. Eight had, in both sera titres of 32 or higher In most of these 8 cases acute-phase serum had been taken later than 1 week after the onset of illness

Only a few specimens were examined for the presence of neutralizing antibodies against adenovirus type 7 It should be

[1] In 3 of the Coxsackie cases significant rise in neutralizing antibodies was demonstrated. From the other 5 cases one serum specimen only was obtained, but all these specimens were found to contain neutralizing antibodies to the isolated virus type.

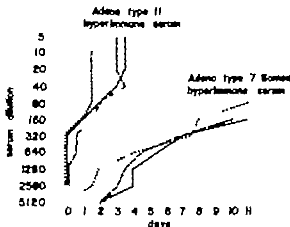


Fig. 1 Neutralization of adenovirus type 7 'Gomen' and type 11 'Slobitski' hyperimmune sera against type 7 'Gomen' type 7A 'S-1058' and strain B 8543/59 isolated during the observed epidemic. Abscissa: duration of inhibition of cytopathic changes. Ordinate: in case value of serum dilution. Three tissue culture tubes per dilution step. ——— prototype 7 'Gomen' received from Dr Huebner 1958 (inoculation dose $10^{4.4}$ $TCID_{50}$). – – – prototype 7A 'S-1058' received from ATCC 1960 (inoculation dose 10^{4} $TCID_{50}$) ...... strain B 8543/59 (inoculation dose $10^{4.4}$ $TCID_{50}$).

mentioned, however that the two patients, who excreted adenovirus type 7 without developing a response in CF titre (<2–<4 2–9) both had neutralizing antibodies against adenovirus type 7 one of them with a rise in titre On the other hand, only one of three patients with titre rise in CF antibodies but negative stool specimen had neutralizing antibodies (and in addition a titre rise) against type 7 Finally rises in CF as well as in type 7 neutralizing antibody titres were demonstrated in 6 out of 1⁹ patients from whom paired sera but no stool specimen were obtained.

The only patient excreting Coxsackie virus who showed rising CF titre (<2-4) against adenovirus had no neutralizing antibodies against type 7

The results of the family studies and of the investigations of the cases with nosocomial infection are summarized in Tables 3 and 4

In none of the 33 controls with scarlet fever could adenovirus be isolated. Nor could a rise in CF titre be demonstrated.

No virus was found in raw water from the water works or in the water of the open-air swimming bath whereas adenovirus type 7 was demonstrated in sewage from an apartment house in one of the suburbs south west of Stockholm and Coxsackie virus type B 3 from another house in the same district.

According to some authors [12], a subtype 7A can be established within the type 7 group The results obtained in our laboratory by repeated neutralization tests agree more closely with those obtained by Binn et al [3], in that only insignificant differences could be demonstrated between the prototype strains 7 7A and three strains from the current epidemic. As an example the results of an experiment with a hyperimmune serum against the Gomen strain are shown in Fig. 1 Analogous results were obtained with another line of the Gomen strain.

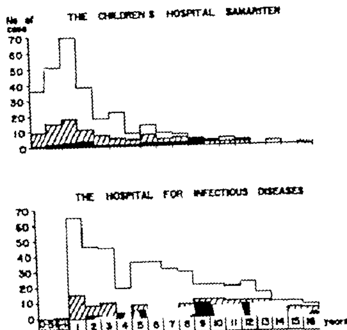


Fig. 2. Age-distribution of 717 children admitted for acute respiratory illness during Aug.–Dec. 1959 ▦ Adenovirus type isolated. ▢ ▨ adenovirus type 7 isolated. ▢ Stool specimen not obtained.

which for control purposes was imported from the American Type Culture Collection in 1900 as well as with another Gomen antiserum which was obtained from Microbiological Associates, Inc In accordance with earlier observations [3 17] hyperimmune sera against types 3 11 and 14 were found to inhibit temporarily the cytopathic effect of type strains This phenomenon was most pronounced for serum against type 11 Fig 1 illustrates the marked difference in slopes between the curves representing heterotypic and homotypic neutralization The prototype strains and three current strains tested behaved identically in this respect

Bacteriological results The bacteriological results are of special interest in those cases where the nose and throat swabs were collected before any antibiotic therapy was started. Within this group one or more of the following bacteria were found in about 50 per cent of the virologically investigated children and about 60 per cent of those not investigated virologically β-haemolytic streptococci, *Staph. aureus* pneumococci nd *Haemophilus influenzae* The frequency of β-haemolytic streptococci was higher in the children who had not been investigated virologically This is explained by the fact that during the late-autumn epidemic of scarlet fever (Fig. 3) in Stockholm a great number of children with streptococcal tonsillitis were admitted to the Hospital for Infectious Diseases, and these were not investigated virologically Among the patients at the Children's Hospital Samariten a higher frequency of pneumococci was found than among those at the Hospital for Infectious Diseases. This difference is possibly attributable to the age-distribution of the patient (Fig 2), since findings of pneumo-

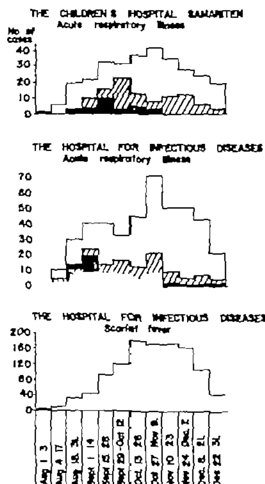


Fig. 3. Number of children admitted with acute respiratory illness or scarlet fever by 14 day periods. ■ Adenovirus type 7 isolated. ▨ No adenovirus type 7 isolated. □ Stool specimen not obtained.

cocci in the nose and throat are reported to be most common in the youngest age-groups [15]. Concerning the other bacteria mentioned no particular differences were disclosed.

One or more of the above-mentioned bacteria was similarly found in about 50% of those 46 children with acute adenovirus type 7 infections, who had not received antibiotic therapy before collection of specimens. The number of cases is too small, however to allow a definite assessment of the frequency of the different bacterial species.

In children who had received antibiotics—in most cases penicillin—before collection of specimens the frequency of the above-named bacteria, *Haemophilus influenzae* excepted, was lower.

From the 253 patients virologically investigated, no Salmonella, Shigella or enteropathogenic *E. coli* were recovered. In the same age-group altogether 10 cases of Salmonella infection, 3 cases of Shigella infection and 1 case of infection with enteropathogenic *E. coli* were diagnosed at the two hospitals during the period under investigation.

Clinical observations. On the basis of clinical symptoms the material can be divided into two main groups: patients with acute respiratory illness, and patients with respiratory illness combined with gastrointestinal symptoms (Table 2).

Of patients investigated virologically 138 belong to the first group. They exhibited symptoms referable to nose and throat, in some cases even to the lower respiratory tract. In 29 of the latter patients bronchopneumonia was diagnosed by X-ray. Symptoms of laryngotracheitis were rare. No conclusions should be drawn from this fact, however, since such patients are as a rule admitted to otological wards at other hospitals.

In the second group consisting of patients who besides symptoms referable to the respiratory tract exhibited gastrointestinal symptoms—diarrhoea with or without vomiting for more than 1 day—84 (including 3 cases of bronchopneumonia) were investigated virologically.

Patients with gastroenteritis—excepting infants—are as a rule admitted to the Hospital for Infectious Diseases. The total number of children with *only* gastroenteritis who were admitted to this hospital

TABLE 2 *Distribution of adenovirus type 7 isolations from cases with different clinical syndromes*

	The Children Hospital Samariten			The Hospital for Infectious Diseases		
	Number of cases			Number of cases		
Symptoms	Total	Investigated	Adenovirus type 7 isolated	Total	Investigated	Adenovirus type 7 isolated
Acute respiratory illness with no signs of gastroenteritis	262	24	19	334	54	17
Acute respiratory illness with gastroenteritis	48	11	4	95	73	49
Gastroenteritis with no signs of acute respiratory illness	19	0	0	_5	5	0

over the period August–December 1959 was only _5 of whom 13 were found to be infected with Salmonella (10 cases) or Shigella (3 cases). On the other hand, 95 children with *respiratory as well as* gastrointestinal symptoms were admitted. Seventy three of these were investigated virologically and adenovirus type 7 was recovered from 49.

Fig. _ shows that adenovirus type 7 was isolated from children of all age-groups including infancy (the youngest patient being 4 months old). The greatest number of strains were recovered from patients at the age of 7–10 years. All the infants except one were admitted to the Children's Hospital Samariten.

The peak incidence of admitted cases occurred in September (Fig. 3).

Most children from whom adenovirus type 7 was isolated were admitted on the third or fourth day of illness.

It has earlier been shown that adenovirus can be excreted for a long time after the onset of infection (9, 10). For this reason the detailed clinical study will be limited to patients with evidence of acut_ adenovirus type _ infection either a po-sitive stool specimen and a rise in titre of CF or type 7 neutralizing antibodies or a rise of both kinds of antibodies but negative or no stool specimen. This makes

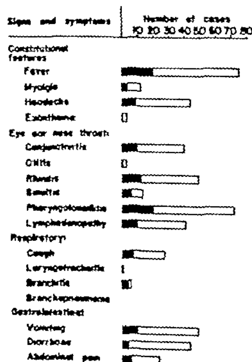


Fig. 4. Clinical features in _5 children with acute adenovirus type 7 infections. ■ = children treated _t the Children Hospital Samariten.

a total of 75 cases from both hospitals including 6 cases of hospital infection in children admitted for some other illness. As seen in Fig 4 the main symptoms were fever conjunctivitis, rhinitis, pharyngotonsillitis cervical lymphadenitis and in more than half of the cases gastroenteritis. An uncharacteristic exanthema was noted in three children In more than four fifths of the cases the fever (≥37 4 C) started with a sudden rise to 39–40 C and lasted for ? to 1? days only two children had fever of less than 5 days duration. The conjunctivitis was often bilateral only in a few cases was it severe and of a purulent nature. The rhinitis was usually fairly mild Nearly all children had pharyngotonsillitis Plugs in the tonsils were often observed also in cases where β haemolytic streptococci were not found, many of these cases had, however received antibiotics before the collection of specimens. In about half of the cases the cervical lymph nodes were moderately enlarged. In a few children the course was complicated by acute otitis media. The diagnosis of sinuitis was made only if pathological changes in the nasal sinuses could be demonstrated radiologically Out of 24 examined children only 13 exhibited such changes of varying degree of severity from thickening of the mucous membranes to complete occlusion Cough was noted in 27 children. X ray examination of the lungs performed in 25 children including five who had bronchitis showed no abnormalities.

Nausea and vomiting were common In more than half of the cases diarrhoea was the predominating feature and led to admission to the Hospital for Infectious Diseases on the suspicion of Salmonella infection. However these children had in addition signs of respiratory infection. Marked abdominal pain was not unusual, whether other symptoms of gastroenteritis were present or not

Many of the children complained of headache, a few of giddiness as well. Muscle pains, mostly in the extremities, were present in some cases. Signs of lesions of the central nervous system were not seen. Examinations of the cerebrospinal fluid in 17 children revealed no abnormalities as regards cell protein or sugar content.

The general condition was in most children good despite the high fever and the period of convalescence was usually short.

Electrocardiograms were taken in 42 children. All but one were normal. The pathological one showed prolongation of the P R interval and was from a child infected with β-haemolytic streptococci in addition to adenovirus type 7

Out of 69 non nosocomial cases 30 had on admission whit blood cell counts below and 10 above the range given as normal for the age [6]. Values below 4000 were recorded in 12 children, whereas only two had counts exceeding 15,000. A slight or moderate increase in neutrophil leucocytes was recorded in most cases. The erythrocyte-sedimentation rat (E.S R.) was determined according to Westergren (in 59 cases) or by Ströms micromethod (in 10 cases). The highest recorded value (mm per hour) varied from normal t 47 (Westergren) and 43 (Ström). In several of the children with a white blood cell count exceeding 10,000 or high E.S.R. or both, bacterial complications could not be demonstrated All the children had haemoglobin values of at least 11 g per 100 ml and normal urine findings.

Among those children from whom adenovirus type 7 was isolated without serological evidence of acute adenovirus infection there also occurred a syndrome of

TABLE 3 *Clinical and virological data among the members of three families in which a child has been found to have infection with type 7 adenovirus on admission to the Children's Hospital Samariten*

\+ obvious; (+) slight; − absent

	N Family					Kj Family				T Family			
Age (years)	7♀	2♀*	30♂	9♂	29♀	2♀*	35♂	33♀	10♂	7♂	5♀	28♀	35♂
Day of onset	Sept. 11	16	23	26	—	Sept. 17	Oct. 6	9	12	Oct. 22	30	30	Nov 2
Max. temp (°C)	40.0	40.4	38.8	39.0	—	40.0	38.5	38 0	—	39 4	40.4	39.5	38.6
Duration of fever (days)	7	13	7	7	−	7	3	3	−	8	5	4	6
Cephalalgia	−	−	−	+	−	+	−	−	−	+	−	+	+
Myalgia	−	−	−	−	−	+	+	−	−	−	−	−	−
Conjunctivitis	+	(+)	+	+	−	+	−	+	−	−	+	+	+
Rhinitis	+	+	(+)	(+)	(+)	+	+	+	+	−	−	−	−
Pharyngotonsillitis	+	+	−	+	−	+	−	(+)	−	+	+	+	(+)
Cough	+	+	+	+	−	−	+	−	−	−	−	−	−
Bronchopneumonia	−	+	−	−	−	−	−	−	−	−	−	−	−
Gastroenteritis	−	−	−	−	−	−	−	−	−	+	−	−	−
Adenovirus type 7	+	+	+	+	+	+	−	+	+	+	−	+	+
CF titre Serum I	2	128	4	4	4	< 2	4	< 2	8	16	16	16	4
Serum II	8	128	4	32	64	4	4	4	8	64	8	16	16

* Hospitalized.

fever conjunctivitis, pharyngotonsillitis and, in many cases, gastroenteritis. This group included 5 cases of pneumonia, in one of which the patient was .. year old girl who had severe atypical pneumonia complicated by myocarditis (see Table 3 family N)

All the children excreting adenovirus types 1 2, 3, 5 and 6 were ill with fever and pharyngitis; one child from whom type 2 was isolated had, in addition radiologically verified pneumonia. One of the children who had adenovirus type 1 in her stools was probably the source of a type 1 infection in another child in the same ward.

The patients from whom Coxsackie virus was isolated had fallen ill with respiratory symptoms. Some had, in addition, muscle pains and mild diarrhoea.

Epidemiology Prior to the outbreak of the epidemic described here 19 adenovirus strains were recovered from children admitted to the Children's Hospital Samariten and the Hospital for Infectious Diseases in Stockholm over the period Jan. 1958–July 1959 (276 investigated cases) Of these strains seven belonged to type 7 and the others to types 1 2, or 5 In July 1959 an adenovirus type 7 epidemic occurred at Nacka a suburb south-east of Stockholm [8].

Other infectious diseases that occurred epidemically in Stockholm in 1959 were influenza and scarlet fever Thus an influenza B outbreak took place during January–March. Absence rates in the schools of up to 25% were noted during January February [1], after which they fell to normal in the last part of March

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Duration of fever (days)	7	13		7	–	7	3	3	–	8	5	4	6
Cephalalgia	–	–	–	+	–	+		–	–	+	–	+	+
Myalgia	–	–	–	–	–	+	+	–	–	–	–	–	–
Conjunctivitis	+	(+)	+	+	–	+	–	+	–	–	+	+	+
Rhinitis	+	+	(+)	(+)	(+)	+	+	+	+	–	–	–	–
Pharyngotonsillitis	+	+	–	+		+	–	(+)	–	+	+	+	(+)
Cough	+	+	+		–	–	+	–	–	–	–	–	–
Bronchopneumonia	–	+	–	–	–	–	–	–	–	–	–	–	–
Gastroenteritis	–	–	–	–	–		–	–	–	+	–	–	–
Adenovirus type 7	+	+	+	+	+	+	–	+	+	+	–	+	+
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Serum II	8	128	4	32	64	4	4	4	8	64	8	16	16

* Hospitalized.

fever conjunctivitis pharyngotonsillitis and, in many cases gastroenteritis This group included 5 cases of pneumonia in one of which the patient was a year old girl who had severe atypical pneumonia complicated by myocarditis (see Table 3 family N)

All the children excreting adenovirus types 1 2, 3 5 and 6 were ill with fever and pharyngitis; one child from whom type was isolated had, in addition, radiologically verified pneumonia One of the children who had adenovirus type 1 in her stools was probably the source of a type 1 infection in another child in the same ward.

The patients from whom Coxsackie virus was isolated had fallen ill with respiratory symptoms Some had in addition, muscle pain and mild diarrhoea.

Epidemiology Prior to the outbreak of the epidemic described here 19 adenovirus strains were recovered from children admitted to the Children's Hospital Samariten and the Hospital for Infectious Diseases in Stockholm over the period Jan. 1958–July 1959 (276 investigated cases) Of these strains seven belonged to type 7 and the others to types 1 2, or 5 In July 1959 an adenovirus type 7 epidemic occurred at Nacka a suburb south-east of Stockholm [8].

Other infectious diseases that occurred epidemically in Stockholm in 1959 were influenza and scarlet fever Thus an influenza B outbreak took place during January–March. Absence rates in the schools of up to 25% were noted during January–February [1], after which they fell to normal in the last part of March

TABLE 4 *Virological data in 19 children with nosocomial acute respiratory illness*

Virus strains isolated from stools	Number of cases	Complement-fixation test against adenovirus
Adenovirus type		
1	1	1/1[a]
2	1	0/1
7	10	5/7[b]
No virus recovered	7	0/5
Total	19	6/14

Numerator: Number of cases with significant rise. Denominator: Number of cases tested.

One case with CF titres < 2 2 had rise in neutralization test against adenovirus type 7

(about 8 %) In the autumn no cases of influenza were recorded. Only sporadic cases of enterovirus infections (polio, Coxsackie and ECHO viruses) occurred in 1959 While the investigation presented here was in progress a large epidemic of scarlet fever broke out It will be seen from Fig 3 that the incidence of scarlet fever reached its maximum somewhat later than did that of the adenovirus type 7 infections

The Hospital for Infectious Diseases receives patients from the whole of Stockholm whereas the Children s Hospital Samariten covers mainly the south western parts of Stockholm and of its surroundings Most of the above-described type 7 cases came from the southern and the western suburbs where the number of large families is greater than in any other residential area of Stockholm. Multiple cases with similar symptoms frequently occurred in the home in the apartment house among playmates in day nurseries in the schools. The family study summarized in Table 3 shows that virtually symptom free infections also occurred.

On the suspicion of hospital infection with adenovirus, 19 children were investigated. Adenovirus type 7 was isolated from 10 (Table 4) Serological results suggested that the infection was contracted in the hospital in at least 6 of these cases. That this type of infection is readily communicable is demonstrated by the following chain of nosocomial infections which also illustrates the difficulty in drawing aetiological conclusions from the clinical picture in acute respiratory illness.

1 *S. record no. 1647/59* A 9 year old boy who on Dec. 6, 1959, became ill with temperature of 40°C, sore throat mild conjunctivitis, vomiting and profuse diarrhoea. Penicillin treatment at home. Admitted on Dec. 10. Nasopharyngeal culture: *H influenzae*. Repeated cultures from stools normal bacterial flora. Stool specimen on Dec. 11 adenovirus type 7 CF antibodies against adenovirus Dec 11 < 2, Dec 19 32.

2. *S record no. 1649/59* A year old boy who was admitted on Dec. 11 the day after patient no. 1 with whom he shared room for the next 24 hours only For few days he had had high fever purulent conjunctivitis and mild diarrhoea. Penicillin treatment at home without effect. Bronchopneumonia, otitis and sinuitis diagnosed on admission. Nasopharyngeal culture: *H influenzae* Afebrile on *Dec. 15 but remained in hospital* for social reasons. Dec. 21 (11th day in hospital): Temperature 39,2°C. Recurrence of conjunctivitis. *Intense rhinopharyngitis*. Loose stools on a few occasions. X-ray of chest on Dec. 29: regression of earlier changes. Nasopharyngeal culture on *Dec. 23: H influenzae*. Stool specimen on Dec 12: no cytopathic agent; Dec. 28 adenovirus type 7 CF antibodies against adenovirus on Dec 12: 2, Dec. 20: < ... Jan. 1960: 8.

3. *S record no 1693/59* A year old girl who had had high spiking fever rhinitis, cough, and conjunctivitis. Admitted on Dec 12. Placed with patient no. 2 in two-bed room. X ray of chest: extensive areas of

bronchopneumonia on both sides Naso pharyngeal culture *H* [illegible] At bride in Dec. 14. Stayed for further investigation [illegible] chest deformity Dec 30 Temp [illegible] 39 [illegible] rhinopharyngotonsill[illegible] [illegible] lymph-nodes N conjunct[illegible] [illegible] mal. Nasopharyngeal culture on Jan 1960 *H* [illegible] and [illegible] Staph. aureus [illegible] specimen on Dec 13 1959 no cytopathic agent [illegible] 3 1960 adenovirus type [illegible] CF [illegible] against adenovirus Dec 1 1959 [illegible] 2 1960 14, Jan 11 [illegible] 64 Neutral[illegible] 3 against type [illegible] Dec 13 1959 [illegible] 2, 1960 negative and [illegible] 11

From these case records it will be seen that patient no 1 probably spread the infection to patient no [illegible] h[illegible] on admission had no adenovirus infection. The patient probably was the source of the infection that patient no 3 cont[illegible]ted during his stay in the hospital

The incubation period [illegible] estimat[illegible] 7 10 days

Discussion

The present [illegible] of adenovirus type [illegible] infection in Stockholm in the autumn [illegible] [illegible] definite conclusion [illegible] cal features [illegible] previous studies [illegible] expects the pi[illegible] with [illegible] virus type [illegible] described [illegible] [illegible] acute [illegible] throat [illegible] largement of the [illegible] the epidemic [illegible] curred in adults [illegible]

During the period of investigation Salmonella and Shigella infection in children occurred to a small extent. On the other hand from children with gastroenteritis in combination with respiratory symptoms adenovirus type 7 was isolated in about two-thirds of the investigated cases. The incidence of isolated adenovirus type 7 strains was [illegible] higher among children with this combination of symptoms than among [illegible] with respiratory infection [illegible] about one third of these investigated [illegible] [illegible]ected that virus type [illegible] infection were demonstrated [illegible] series consisting of patients [illegible] for a period of time [illegible] connected with the length of [illegible] epidemic and from [illegible] Stockholm

It seems therefore [illegible] adenovirus type 7 [illegible] respiratory symptoms [illegible] gastroenteritis [illegible] findings [illegible] type 3 may also [illegible] symptoms [illegible] be well that [illegible] infection may [illegible] The high incidence [illegible] infections among [illegible] particularly [illegible] tal areas [illegible] from where [illegible] The spread of [illegible] the families [illegible] tions suggest [illegible] closed group [illegible] during the [illegible] was, however [illegible] instance [illegible]

and did not exceed the average morbidity among school-children at this time of the year [7]

Modes of transmission other than personal contact, for instance via children's paddling pools public baths, etc are possible but have not been established by evidence

Summary

During an epidemic in Stockholm in the autumn of 1959 adenovirus type 7 was isolated from 99 hospitalized children. There was serological evidence of acute adenovirus infection in 75 cases. The virus strains could not be referred to types 7 or 7A according to the scheme of Rowe et al., since no definite differences between the prototypes for these strains could be demonstrated by cross-neutralization tests. In addition to the symptoms of acute respiratory illness observed previously in such infections many of the patients exhibited symptoms of gastroenteritis, for which no other cause could be established.

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References

1 Skoloverläkarens for Stockholm Årsrapport för 1958–1959 bilaga 1
2. Bahr, J Kjellén L. and Svedmyr, A.: Hospital outbreak of adenovirus type 3 infections. *Acta Pædiat 47* 265, 1958.
3. Bell L. K Hilleman M. R., Rodriguez, J E. and Glabere, R. R. Antigenic relationships among adenoviruses with appraisal of reliability of complement fixation test for typing isolates. *J Immunol 80* 501 1958
4. Chany C. Lépine, P Lelong, M., Le-Tan Vinh, Satgé, P and Virat J Severe and fatal pneumonia in infants and young children associated with adenovirus infections. *Amer J Hyg 67* 367 1958.
5. Fanconi, G. and Wallgren A. Lehrbuch der Pädiatrie. Benno Schwabe & Co, Basel/Stuttgart 1961
6. Fulton F and Dumbell, K R. The serological comparison of strains of influenza virus. *J Gen Microbiol 3* 97 1949
7 Hjärne, U Personal communication.
8. Kallings, L. O and Madsen B To be published.
9 Kjellén L. Sterner G and Svedmyr, A.: On the occurrence of adenoviruses in Sweden. *Acta Pædiat 46* 164 1957
10. Kjellén L. Zetterberg B and Svedmyr, A A epidemic among Swedish children caused by adenovirus type 3. *Acta Pædiat 46* 561 1957
11 Oker Blom N. W... a O Strandström, H., Mäkelä, P and Jansson E.: Adenoviruses associated with pharyngoconjunctival fever *Ann Med Exp Fenn, 35*, 342, 1957
12. Rowe W P., Hartley J W and Huebner, R. J.: Serotype composition of the adenovirus group *Proc Soc Exp Biol Med 97* 465, 1958.
13. Sterner, G Infections with adenovirus type 7 in children and their relationship to acute respiratory disease. *Acta Pædiat. 48* 287 1959
14 Svedmyr, A., Enders, J F and Holloway A.: Complement fixation with the three types of poliomyelitis viruses propagated in tissue culture *Amer J Hyg 57* 60, 1953.
15. Tunevall, G Oto-rhinological infection in childhood. *Acta Pædiat 41* Suppl. 92. 1952.
16. Tyrrell, D A. J Balducci, D and Zaiman T E Acute infections of the respiratory tract and the adenoviruses. *Lancet, 271* 1326, 1956.
17 Uchida, S. Hoshika T Yamamoto, H., Kondo, K. Konishi, S and Fujino, A.: Relationships between types of adenovirus indicated by heterologous neutralization. *Jap J Exp Med 29* 121 1959.
18. v Zeipel, G and Svedmyr, A.: A study of the association of ECHO viruses with aseptic meningitis. *Arch Ges Virusforsch. 7* 355, 1957

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The Bacterial Flora of the Upper Respiratory Tract and Gut in Children of Nomad Lapps[1]

by GUNNAR LAURELL and TORE MELLBIN

The nomad Lapps differ in certain respects from the rest of the population in Sweden. They are constantly on the move, following the reindeer herds high up into the mountain districts during the summer and down towards the coast in winter and the families therefore have to have at least two, and often more, dwelling places. During the summer these people are isolated in the mountains: in winter they usually live in small inland villages. Compared with the rest of the population, therefore, they are in most respects more isolated all the year round. This isolation has been reduced during recent years, especially in the southern regions, but in the most northerly districts it is still marked.

Like other children in Sweden, the nomad children begin school at 7 years of age Owing to the great distances they go to boarding schools, where they are in close contact with each other in contrast to the time before they start school and to the summer holidays.

The contact that exists between the nomad school-children and the sedentary population varies in the different school districts and is closer in the more southerly of those investigated. The children from the northernmost districts are therefore more isolated during both term and holidays, and also before they start going to school.

The life of the Lapps is, as we have said, conditioned by the movements of the reindeer The proximity of the people to these animals and their produce and the abundance of dogs might be expected to be reflected in the bacterial flora of the people.

With regard to these conditions, investigations were carried out to discover the nature of the bacterial flora of the nomad children in connexion with other investigations on these children (Mellbin, to be published)

An investigation such as this is also of interest in a wider sense In these times there are some difficulties in finding material to make up a normal series. This applies not least to the bacteria of the respiratory tract. The widespread use of antibiotics has produced a shift in the bacterial flora, sensitive organisms being eliminated and replaced by resistant sometimes of the same species but commonly of another This trend is most

[1] This investigation was supported by grant from the County Council of Norrbotten, Sweden.



Fig. —. Pathogenic bacteria. Percentage incidence in nose and throat among children from the various schools. Schools: K. = Karesuando, L. = Lannavaara, Ju. = Jukkasjärvi, G. = Gällivare Jo. = Jokkmokk, A. = Arjeplog

Kauffman enrichment medium for isolation of Salmonella. 12, Desoxycholat citrate gar plates for Salmonella and Shigella.

Plates 1 3 4, and 5, and tubes 6 and 7 were incubated in air at 37°C and were read off after 48 hours. Plates 3 and 5 were then incubated for a further 3 days at room temperature and again read off. The gentian violet plates were incubated in air for 48 hours. Secondary culture was carried out from tubes 6 and 7 if no growth was obt ined on the primarily inoculated plates.

When reading off cultures from the air passages ll suspected *Staph. aureus* β-haemolytic Streptococci, Pneumococci, *H influenzae*, and fungi were noted The suspect colonies were then examined as follows.

Staphylococci. The coagulase test was carried out, and all coagulase-positive strains, irrespective of pigment formation, were subsequently referred to as *Staph. pyogenes* Most strains obtained at the first period of investigation were phage-typed by Dr Gösta Wallmark, at the National Bacteriological Laboratory Stockholm. One hundred and fifty five strains obtained on the same occasion were also tested for formation of α β- and δ-lysin, using the technique worked out by Elek & Levy (2)

Streptococci were tested on soluble haemolysin. Strains forming free haemolysin were grouped and typed by Dr Ingmar Juhlin, at the Department of Bacteriology Allmänna Sjukhuset, Malmö.

Pneumococci were typed by Neufeld's capsular swelling reaction. Untypable Pneumococci were confirmed by means of optochin (strip method)

H influenzae were confirmed by means of the satellite phenomenon.

Fungi. Isolated fungi were obtained in pure culture, and typed in the usual manner by means of staining techniques, culture on spore substrate, and biochemical fermentation.

The examination of the faeces included standard tests for Salmonella and Shigella organisms. Pathogenic *E coli* were selected via Conradi Drigalski medium, and were confirmed by standard serological tests. *Staph. pyogenes* were isolated from phenol-mannite plates.

Sensitivity tests

All strains f *Staph. pyogenes* recovered at the first period of investigation were tested by the disk method (3). The limits between sensitivity and resistance were set as follows: penicillin, 2 I.U./ml, erythromycin, 4 μg/ml, streptomycin, 12 μg/ml; tetracyclines, 4 μg/ml; chloramphenicol, 8 μg/ml, novobiocin, 16 μg/ml. In the description that follows,

TABLE 1. *Children with positive throat and/or nasal culture at the various schools during the two periods of investigation (1957 after 7 weeks at school 1958 beginning of term) Percentage incidence.*

School	Karesuando	Lannavaara	Jukkasjärvi	Gällivare	Jokkmokk	Arjeplog	Total
			First period of investigation, 1957				
No. of children	46	67	68	64	56	29	330
Staph. pyogenes	50	61	66	78	73	66	69
β-haemolytic Streptococcus	3	8	10	36	23	28	17
Pneumococcus	15	18	24	33	63	48	33
Haemophilus influenzae	0	0	7	20	12	0	8
Fungi	7	6	10	13	12	14	10
			Second period of investigation, 1958				
No. of children	46	63	60	49	61	33	310
Staph. pyogenes	74	64	78	61	70	91	76
β haemolytic Streptococcus	4	3	0	2	2	6	3
Pneumococcus	43	50	28	24	21	38	34
Haemophilus influenzae	11	8	2	6	3	0	5
Fungi	43	24	35	37	36	22	33

strains that were inhibited by these concentrations are termed sensitive, and the rest resistant.

Serological tests

Antistreptolysin titres (AST) were determined chiefly as described by Ipsen (5). The results, however are given in the manner proposed by Packalén & Bergqvist for antistaphylolysin (ASta) determinations (6).

Antistaphylolysin titres (ASta) were determined in accordance with the directions of Packalén & Bergqvist (6)

Results

Upper Respiratory Tract

Staph. pyogenes

Incidence

Fig _ shows the incidence of *Staph. pyogenes* in throat and nose among the children at the various schools during the first period of investigation. The total percentage incidence of this organism in

TABLE _. *Percentage incidence of Staph. pyogenes Pneumococcus and fungi among first-form children on starting school and after 7 weeks at school. The corresponding figures for the whole series 1957 and 1958 are included for purposes of comparison*

Organism	First form 1957: on starting school, 53 children	First form 1957: after 7 weeks at school, 48 children	Total series: 1957 330 children	Total series: 1958 310 children
Staph. pyogenes	63	60	69	76
Pneumococcus	31	40	33	34
Fungi	53	13	10	33

TABLE 3 *Staph. pyogenes. Phage patterns among 302 strains isolated from nose and throat during the first period of investigation*

Phage group	Phage pattern	First form on starting school, 55 children	Total series after 7 weeks at school, 220 children
I	52/52A/K86	1	10
	52/52A/166	0	2
	52/52A/80	2	2
	K86	0	21
	52/80	2	1
		5	*36*
II	3A	3	12
	3B/3C/55/71	6	15
	3B/3C	0	1
	3C/55	0	3
	3C/55/71	0	3
	71	6	46
		15	*81*
III	6/47/53	0	4
	6/47/54/75/819/1034+	2	9
	53	0	3
	53/54	0	3
	73	4	3
	77	0	3
	Further 16 patterns	5	19
		11	*44*
IV	42D+	1	1
		1	*1*
I + III	52A/79/53/73/819	0	4
	Further 2 patterns	1	2
		1	*6*
	166/185	1	20
	185	0	7
		1	*27*
Untypable		32	41
Total number of strains		*66*	*236*

the respiratory passages is illustrated in Table 1. *Staph. pyogenes* was isolated from 69 % of all the children, the local figures showing considerabl variations (from 59 % in Karesuando to 86 % in Arjeplog). The incidence of Staphylococci after the period of 7 weeks at school was thus lower in the more northerly schools than in those farther south. This difference is significant.

Scrutiny of the figures for the incidence of Staphylococci among school starters (see Table 2), from whom swabs were taken both at th beginning of term and 7 weeks later, reveals that 93 % of these children had Staphylococci in the nose and/or throat on the first occasion, whereas the corresponding figure at 7 weeks was 60 %. Because the series is so small (55 children) it cannot be divided

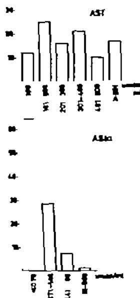


Fig. 1. Antistreptolysin (AST) and antistaphylolysin (ASta) titres. Percentage distribution.

up into the respective school groups, but 31 of the children that had Staphylococci at the beginning of term still had these organisms, 20 had lost them, and 3 that had previously been negative now after 7 weeks, had acquired them. Two children showed negative cultures on both occasions.

At the 1958 examination (see Table 1) all specimens were taken at the beginning of term. The overall incidence of *Staph. pyogenes* was then 76 %, and there was no clear difference between the northern and southern schools.

Sensitivity tests

Altogether 304 strains were tested. The incidence of strains resistant to most antibiotics was extremely low amounting to 0.8 for erythromycin, chlortetracycline, oxytetracycline, tetracycline and chloramphenicol, and to 1.1 % for streptomycin. The incidence of strains resistant to penicillin was 7.9 %, and to novobiocin 5.5 %. The figure concerning novobiocin is noteworthy especially as it was found that 29 % of the Staphylococci strains isolated from the school starters at the beginning of term were resistant to this substance.

Phage typing

A total of 302 strains were examined, 66 from children in the first form at the beginning of term and 236 from all children 7 weeks after the beginning of term. The results are shown in Table 3. Phage typing was on the whole successful, especially in the case of strains isolated 7 weeks after the commencement of term. Of these latter 236, only 41 proved to be untypable, whereas the corresponding figures for the first form children at the beginning of term were 3_ out of 66 strains, i.e. 48 %. After 7 weeks at school 32 strains of *Staph. pyogenes* were isolated from the first formers, and of these only three (9 %) were untypable. The typable strains were chiefly referable to groups I, II, and III, with nearly twice as many in group II as in any of the other three groups. It is particularly interesting that no fewer than 52 strains belonged to phage type 71. Apart from the higher incidence of untypable strains, there were no differences concerning distribution between strains isolated at the beginning of term (school starters) and those isolated 7 weeks later (whole series).

Haemolysin pattern

Altogether 163 strains of Staphylococci were examined, and the results are given

TABLE 4 *Staph pyogenes Haemolysin pattern for 155 strains isolated during the first period of investigation*

Haemolysin pattern	Number of strains	Percentage distribution	Elek & Levy percentage distribution of [illegible]00 strains
αβδ	26	17 } 18	11
βδ		1 }	—
αδ	105	68	82
	9	6	3
δ	13	8	4
Total	155	100	100

in Table 4 It is of interest that 18 % showed β haemolysis which is more than what is usually found in Staphylococci isolated from man.

Antistaphylolysin (ASta) determinations

Serological examination (see Fig 3 and Table 5) disclosed no important incidence of raised ASta titres The titre in no fewer than 92 % was 1 4 U or less. Concerning the raised titres 7 % of the children had titres between 1 4 and 2.8 U., and only 0.9 % exceeded 2.8 U There was no significant difference between the various schools or between the age groups. The mean value for the series was 0.85 U (limits 0 74 U in Jokkmokk and 0.98 U in Karesuando)

β-haemolytic Streptococci

Incidence

During the first period of investigation Streptococci were isolated from 57 children (17 %). As can be seen from Fig   and Table 1 the variations were fairly great. In the two northernmost schools these organisms were isolated in only [illegible] % and 8 % of children respectively whereas the incidence in Gällivare was 30 % and in the two southernmost schools 23 % and 25 %. They were chiefly isolated from the throat, but nasal carriers were also found. The incidence of the organism among school starters at the beginning of term was 9 %. During the second period of investigation the overall incidence of β-haemolytic Streptococci was only 3 %, with isolated cases in five of the six schools

TABLE 5 *Antistaphylolysin and antistreptolysin titres Mean values at the different schools*

School	Karesuando	Lannavaara	Jukkasjärvi	Gällivare	Jokkmokk	Arjeplog	Total
No. of children	46	67	68	64	56	49	350
Antistaphylolysin (ASta), mean titres	0.96	0.83	0.90	0.84	0.74	0.83	0.85
Antistreptolysin (AST), mean titres	348	375	484	395	[illegible]	199	364

Typing

The results can be seen in Table 6 Typing could be carried out satisfactorily and most of the strains belonged to Lancefield's group A. Other groups were represented by only a few strains. Type 9 was commonest (19 strains) followed by types 6 and 10 It is of interest that four strains belonged to type 12, a type ascribed importance in connexion with nephritis.

TABLE 6 *Streptococcus Type-distribution among 69 strains isolated from nose and throat during the first period of investigation.*

Type	First form on starting school, 55 children	Total series after 7 weeks at school, 330 children
Group A		
6	0	8
9	1	18
10	0	8
12	1	3
14	0	2
21	1	5
27	0	1
28	0	2
Group B	0	1
Group E	0	1
Group F	0	2
Group G	1	1
Group L	0	3
Untypable	0	6
Not typed	1	3
Total no. of strains	5	64

Antistreptolysin (AST) determinations

The results of serological analysis are collected in Fig. 3 and Table 5 A surprisingly large number of high titres were noted, no fewer than 90 % exceeding 400 U There were fairly large variations between the schools, with significantly lower mean values for the 85 children from the two southernmost schools despite the higher incidence of Streptococci at these If the titres are arranged according to age groups it is found that the mean titres increase over the three lowest forms (286 329 367), and then remain roughly constant at the high mean level of 398 No correlation between these figures and the current incidence of β-haemolytic Streptococci could be established.

The AST values for 54 school-starters were rather higher at the beginning of term (mean 310) than after 7 weeks at school (mean 296) After this period the titres for 30 children had fallen, 13 were unchanged, and 11 had increased.

PNEUMOCOCCI

Incidence

The incidence of Pneumococci during the first period of investigation was 33 % (see Fig 2 and Table 1) Here again there were considerable variations between the schools, the two northernmost showing the lowest values (15 % and 18 %, respectively) and the two southernmost showing the highest figures (68 % and 48 %, respectively). At the beginning of term Pneumococci were present in 31 % of the school starters, and 7 weeks later in 40 % (see Table 2)

During the second period of investigation the incidence was 34 %, the variations from school to school were less, and no statistical difference could be found between the northernmost and southernmost schools.

Typing

Typing of Pneumococci was carried out during the first period of investigation, and here again most strains were typable (only 5 % proved to be untypable). No

r d minating type wa t L Type 19 wa commonest f llowed l y types 23 18 and 30

The typability wa among strains isolated from school starters at the beginning of rm Of l it was possible to typ only whereas all str isola l am children later l

Hae fl enza

isolated during the ation in 8 of all and Table 1) The great and in several was not present at all. nd occasion the overall inci and again the variations gr t ping wa not undertaken.

Fungi

In id nce

During the first period of investigation fungi were isolated from 10 of the children after 7 weeks at school (see Table 1) The incidence was lowest in Karesuando and increased southwards to a maximum of 14 (Arjeplog) Specimens taken from school starters at the beginning of term (see Table) showed that 53 of the children then had fungi in throat and/or nose whereas the incidence of these organisms in the same children 7 weeks later was 13 an incidence of the same order of magnitude as that for the larger series. This difference is highly significant

During the second period of investigation the incidence of fungi was 33 with fairly even distribution between the various schools and age groups (except for Forms 1 and 2, in which the figures were 50 % and 40 respectively)

Typing

During the first period of investigation typing of 60 strains was carried out. Of these 43 were *Candida albicans* 6 Geotrichum 4 Torulosis 3 Trichosporon, 2 non pathogenic Cryptococcus, 1 *Candida parakrusei* and 1 Rhodotorula

Faeces Culture

Incidence of organisms

No Salmonella or Shigella bacteria were isolated, but *Staph pyogenes* and pathogenic *E coli* were present The findings are collected in Table 7 *Staph pyogenes* was isolated in 41 cases, i.e 1 and was commonest in Jokkmokk. Pathogenic *E coli* were isolated in eight cases. In five of these the strain was 0111 B4 and the remaining three were 0155B15 0126B16, and 0112a 011 c.

Transport Medium

Having regard to the special interest attached to an improved transport medium a new substrate previously used chiefly for Gonococci was tried, first tentatively and during the second period of investigation to a greater extent. The results are shown in Table 8 As can be seen the medium offers certain advantages in the case of th most sensitive organisms, namely *Haemophilus infl enzae* No particular advantages were apparent with regard to other bacteria. It is worthy of note that fungi were isolated more than twice as often with ordinary agar tubes as when the special substrate was used as

TABLE 7 *Faeces culture Distribution of positive cultures among the various schools*

School	Karesuando	Lannavaara	Jukkasjärvi	Gällivare	Jokkmokk	Arjeplog	Total
No. of children	46	6	65	64	56	29	330
Staph. pyogenes	6	9	1	6	16	3	41
Pathogenic E. coli	0	1	4	2	0	1	8

transport medium. The same trend was apparent though less markedly in the case of *Staph pyogenes*.

Discussion

The incidence of *Staph. pyogenes* was surprisingly high during both periods of investigation, viz. 69 and 76 %, respectively and an incidence of as much as 93 % is recorded for the school starters. As rule the incidence of Staphylococci outside hospital is about 40 %, and in 1954 among the natives of New Guinea it was only 19 %.

Striking too is the fact that the incidence of Staphylococci is greater among children arriving at school from the isolated home environment than after 7 weeks life at school in close contact with other children in classrooms and dormitories. The high incidence in these children at the beginning of term may be connected with their proximity at home to domestic animals, from which these organisms may originate When they leave the home environment the incidence of Staphylococci falls.

On phage typing it was found that 48 % of the school-starters Staphylococci at the beginning of term were untypable whereas the corresponding figure after 7 weeks was only 9 %. This would support

TABLE 8 *Distribution of positive and negative cultures of the organisms concerned using different media.*

Organism	Culture	First period of investigation Throat Agar	First period of investigation Throat Special	Second period of investigation Nose and throat Agar	Second period of investigation Nose and throat Special
Staph. pyogenes	Negative	2	40	40	[illegible]
	Positive	45	40	323	220
β-haemolytic Streptococcus	Negative	6	1	4	3
	Positive	9	14	3	6
Pneumococcus	Negative	2	5	40	42
	Positive		4	[illegible]	73
Haemophilus influenzae	Negative	3	6	1	3
	Positive	0	5	8	12
Yeasts	Negative	0	9	12	61
	Positive	10	1	93	46

the theory that the Staphylococci originate from domestic animals.

It is of interest that many of the strains belonged to phage-type 71, a type described as particularly common in impetigo among children. Oddly enough, it is not especially common in the respiratory passages of children with this infection. There was no case of impetigo among the children that were carriers of this phage-type.

The incidence of β haemolysis among the isolated Staphylococci was 18%, which is higher than that usually noted in Staphylococci isolated from man. β-haemolysis is commoner among Staphylococci from animals, on the other hand (2).

Despite the high incidence of *Staph. pyogenes*, the antistaphylolysin titres were within normal limits in 92% of the children, and only 0.9% showed undoubtedly raised titres. The ASta titres were of the same order of magnitude at the beginning of term and 7 weeks later in the school-starters.

The organisms showed marked sensitivity to most of the antibiotics tested. Of the strains 7.0% were resistant to penicillin, and the incidence of strains resistant to novobiocin was 5.5%. The high figure for penicillin may be due to previous treatment with this substance. It is known that a resistant strain may persist long after it first invaded the respiratory passages. The novobiocin-resistant strains are not so easily explained, since this antibiotic has with absolute certainty never been used among this population. There may be some connexion with the fact that these Staphylococci to some extent originated from animals. It is not known whether animal Staphylococci have a naturally higher resistance to novobiocin, but the fact that 20% of the strains isolated from the school-starters at the beginning of term were resistant to novobiocin would be in favour of this.

Whereas the incidence of Staphylococci during the second period of investigation (at the beginning of term) was fairly even at the various schools, it is found that during the first period of investigation, which took place 7 weeks after the beginning of term, the incidence increased the farther south the school was situated. As mentioned in the introductory paragraphs, the more southerly schools are less isolated from the sedentary population than are those in the north. The higher incidence of Staphylococci in the more southerly districts may therefore be due to closer contact with carriers from outside the schools.

The incidence of *β-haemolytic Streptococci* during the first period of investigation varied greatly from one school to another. The reason for this is probably cross infection among the children during the 7 weeks they had been resident at school before the swabs were taken. The lower incidence at the beginning of term both among school-starters during the first period of investigation and in the total series during the second (9% and 3%, respectively) would support this assumption. Whether the spread within the groups may be explained by the presence of carriers among the children themselves or by infection from members of the sedentary population it is impossible to say, but it is a fact that the incidence of Streptococci is much lower in the northern more isolated schools.

Typing yielded nothing of special

Fig. 4. Percentage incidence of infection with one or more of the pathogens demonstrated during the two periods of investigation. Schools: K. Karesuando, L. = Lannavaara, Ju. = Jukkasjärvi, G. Gällivare Jo. = Jokkmokk, A. = Arjeplog.

interest, apart from the fact that four strains belonged to type 12. This is regarded as particularly liable to cause nephritis, but no clinical signs of this condition or of rheumatic infection were seen.

It is difficult to find a satisfactory explanation for the unexpectedly high anti streptolysin titres, which exceeded 400 U in 28% of cases. The rise in titre with increasing age is in accordance with the findings in earlier investigations. The high titres among the children from the more northerly schools may possibly be due to the lower social standard prevailing in those districts. Owing in part to the poor housing conditions respiratory infections are common, especially during winter before the children even start school and in school holidays.

Pneumococci. Pneumococci were common, and were isolated to the same extent during both periods of investigation. As in the case of Staphylococci, the incidence was higher in the more southerly schools at the first examination but on the second occasion the distribution was evener. The typability appeared to be poorer among the strains introduced into the environment by the children themselves than among those isolated 7 weeks after the beginning of term.

The incidence of *Haemophilus influenzae* was low on both occasions, and varied greatly from school to school. No conclusions can be drawn from the figures available. It is however of interest to note that it was at all possible to isolate these comparatively sensitive organisms in spite of the long time that elapsed between sampling and culture.

The influence of carriers of infection among the sedentary population upon the children residing in boarding schools is indicated in Fig 4. The percentage incidence of infection with one or more of the pathogens demonstrated in nose and throat after 7 weeks at school shows an obvious increase from north to south, which clearly reflects the degree of contact between the school-children and the rest of the population. For purposes of comparison, the corresponding figures for the second period of investigation are included. At that time the children had just returned from a stay of about 3 months duration in the mountains. The high overall incidence of bacteria on this occasion is explained by the higher incidence of Staphylococci among the children at the beginning of term (see Table 1).

The incidence of *f ngi* in swabs taken immediately on the children's arrival at

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Immune Electrophoretic Studies of Bovine Milk and Milk Products

by LARS Å. HANSON and I MÅNSSON

A large number of antigenic substances have been demonstrated in bovine milk by means of immune electrophoresis (5 6 8) Some 10–15 of these antigenic factors are related to blood serum proteins among others to the albumin and the γ-globulin Several of the specific milk proteins have been identified with chemically characterized proteins such as α lactalbumin β lactoglobulin casein and red protein The immunological techniques provide a more descriptive and complete picture of the milk proteins than does for example simple electrophoresis. For this reason we have used the immune electrophoretic technique to study the changes of the proteins which may be induced by various methods employed in the commercial preparation of milk and milk products The possibility of using this immunological method for the analysis of milk allergies is also taken into consideration.

Material

Antigens

Bovine colostrum taken two hours after parturition was used as well as mature milk taken more than three weeks after parturition. Merthiolat was added t a concentration of 1/10 000 These materials were also examined after heat treatment for 15 minutes at 55° 60° 65° 70 75 80° 85° 90° 95 100° and 1*0°C Mature milk was analyzed after being subjected to the following pasteurization procedures: 63°C for 30 minutes, 75°C for 10 seconds and 90°C for 120 seconds.

Dried milk prepared by various methods[1] (spray-dried single and double drum-dried) was examined as well as samples of commercially available baby food products[2] containing milk, spray-dried after pre-heating to 84°C. A commercial preparation of bovine milk advertized as "non-allergenic"[3] and intended to be given to infants hypersensitive to bovine milk was also tested. For the analyses the milk powders were dissolved in the phosphate buffer (pH 8.4) used for the electrophoresis.

Immune sera

Anti-colostrum immune sera were prepared in rabbits by weekly injections of 2 ml of colostrum for 4 weeks. After this time they were bled Other bleedings were taken one week after subsequent booster doses. Comparison of immune electrophoretic analyses of milk with the anti-colostrum sera and with anti mature milk immune sera showed mainly identical spectra. The anti-colostrum

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[1] Kindly supplied by Eng Thorsell, VMC, Vänersborg (Sweden).

[2] "Semper Välling and Baby Semp kindly supplied by AMP Stockholm (Sweden).

[3] "Allergilac Cow-lac Ltd., London (England).

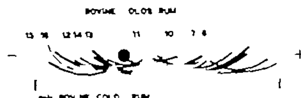


Fig. 1 Diagrammatic representation of an immune electrophoretic analysis of bovine colostrum by means of anti-colostrum immune serum. The following precipitation lines are identified 2 and 3, casein; 4, β lactoglobulin; 5, serum albumin; 7 -lactalbumin; 8 red protein and 12 immune globulin component.

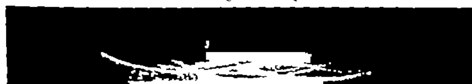


Fig. 1b Photograph of the analyses drawn in Fig. 1

TABLE 1 *The heat stability of bovine colostrum proteins as determined by immune electrophoresis*

+ indicates presence of line ( ) indicates presence of fuzzy line

Time min.	Temp. °C	Line number (see Fig. 1) 1	2	3	4	5	6		8	9	10	11	12	13	14	15	16*
15	60		+	+	+	+	+		−	−			+	+		+	
15	65		+	+	+	+		−	+	+		+	+	+	+	+	
15	70		+	+	+			−	+	(+)		+	(+)	(+)		+	
15	75		+		−	+		+	( )			+				+	
15	80		+	+	(+)			−	(+)			+				+	
15	85		+		(+)			+	(+)			+				+	
15	90		+		(+)			+	(+)			(+)				+	
15	95		+		(+)			+								(+)	
15	100		+		(+)			−								(+)	
15	120		+														

Lines 1 and 16 are seen only inconsistently in the colostrum–anti-colostrum spectra.

sera, however gave rise to more distinct precipitates permitting more ready interpretation of these spectra. Therefore only such immune sera were used in the present investigation.

Method

The analyses were performed with the immune electrophoretic method of Grabar & Williams (3, 4) in the modifications described by Wadsworth & Hanson (15).

Results

Immune electrophoretic analysis of bovine colostrum by means of anti-colostrum immune serum showed a spectrum of at least 16 precipitates. Their shape and localization are shown in Figs. 1a and 1b Some of these precipitates have been identified with known milk and blood serum proteins (6, 8) as indicated in Fig. 1a. The heat-sensitivity of the proteins



Fig 2 Photograph of n immune lectrophoretic analysis of colostrum heated to 1°0°C for 15 minutes. Immune serum ant -colostrum. Only the casein precipit tes (nos. and 3) are seen. Anode t the right

corresponding to the immunoprecipitates is illustrated by the results of the immune electrophoretic analyses listed in Table 1 It may be seen that exposure to heat for 15 minutes resulted in little change in the antigenic reactivity until the level of 70 – 75°C was employed At this temperature the major immune globulin component among others was affected (line no. 1°) Line no 15 in the immune globulin region persisted however even after exposure to 100°C The heat resistance of casein (nos. and 3) α lactalbumin (no 7) and β lactoglobulin (no 4) was notable After treatment at 120°C only the casein precipitates were obtained (Fig)

Immune electrophoretic analyses of mature milk showed a spectrum consisting of 1° precipitation lines (Fig 3) Some of these were designated since they were identifiable with known milk proteins. Nine of them identified with precipitates in the colostrum spectrum and were given the same numbers as used in that spectrum (Fig. 1a). One precipitate was seen only inconsistently and was not included in this report, while two others in the immune globulin region could not with certainty be identified with the corresponding precipitates in the colostrum spectrum they were therefore given separate numbers (nos. 17 and 18) in Fig 3 All of these precipitation lines were still seen after heating the milk to 70°C for 15 minutes

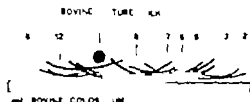


Fig. 3. Diagrammatic representation of an immune electrophoretic analysis of bovine mature milk by means of anti-colostrum immune serum. The numbers indicat the same precipitates as in the colostrum spectrum (Fig. 1). Precipit tes numbered 17 and 18 have not with certainty been identified with the corresponding precipitates in the colostrum spectrum. Anode t the right.

(Table) however a temperature of 75°C changed the immune globulin component which gave rise to the dense precipitate found in this region (no. 1). The serum albumin line in the mature milk spectrum was still apparent as a fuzzy precipitate even after exposure of the milk to 100°C. In colostrum no such precipitate was seen after heating to 75°C With the exception of this precipitate similar results were noted for colostrum and mature milk after exposure to 100 and 120°C As can be seen in Fig. 4, milk was also analyzed after pasteurization by different methods heating to 64 C for 30 minutes 75°C for 19 seconds and 90°C for 120 seconds. It was quite evident that heating to 64°C for 30 minutes caused only minor changes of the proteins while the higher temperatures had a more deleterious effect (Table 2).

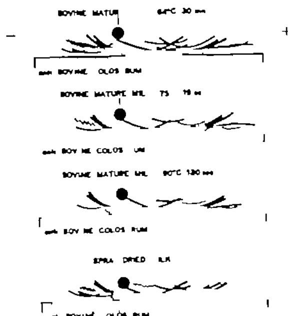


Fig. 4. Diagrammatic representations of immune electrophoretic analyses of pasteurized and spray-dried milk by means of anti-colostrum serum.

The analyses of dried milk showed that spray-drying (Fig. 4) was somewhat less harmful to the proteins than was drum drying (T ble 2) After double drum drying the same degree of denaturation of the milk proteins was seen as for the baby food products examined (Table 2)

The non-allergenic milk product for children hypersensitive to milk showed retained antigenicity of the substances forming the casein, the α lactalbumin and the β-lactoglobulin precipitation lines (Table).

Discussion

Since the beginning of this century several investigators have studied and discussed the effect of heat on bovine milk proteins on the basis of results obtained with various immunological techniques (10 11 1 and others) Electrophoretic techniques have also been applied in such studies (2, 9 14) With the aforementioned methods it has only been possible to study a few fractions of milk or isolated proteins. By means of immune electrophoresis a descriptive and more complete picture is obtained of the changes of the milk proteins. In addition it is unnecessary to work with isolated purified substances since each substance may be analyzed individually as a separate precipitate in the immune electrophoretic spectrum. This is also advantageous as different heat-sensitivities have been observed for isolated milk proteins and for these same proteins in whole milk (9 10) The actual experiments were planned to illustrate the changes detectable in processing milk. It was demonstrated that casein resisted 120 C and that α lactalbumin and β lactoglobulin were still

TABLE 2 *The heat-stability of bovine mature milk and milk products as determined by immune electrophoresis*

+ indicates presence of line; (+) indicates presence of fuzzy line

Material	Time min.	Temp. C	Line number (see Figs. 1 and 3) 1		3	4	5	6	7	8	1	17	18
Mature milk	15	60	+	+	+	+	+	+	+	+	+	+	+
	15	65	+	+	+	+	+	+	+	+	+	+	+
	15	70	+	+	+	+	+	+	+	+	+	+	+
	15	75	+	+	+	+	+	+	+	+		+	+
	15	80	+	+	+	+	+	+	+	+		+	+
	15	85	+	+	+	+	(+)		+	+		+	+
	15	90	+	+	+	+	(+)		+	+		+	+
	15	95	+	+	+	+	(+)		+	+		+	
	15	100		+	+	+	(+)		+			+	
	15	120		+	+								
Pasteurized milk	30	64	+	+	+	+	+	+	+	+	(+)	+	+
	19 sec	[illegible]5	+	+	+	+	+		+	+	(+)	+	
	1[illegible]0 sec	90	+	+	+	+	(+)		+	+		+	
Spray-dried milk			+	+	+	+	+	(+)	+	(+)	(+)	+	+
Single drum-dried milk			+	+	+	−	+	(+)	+	(+)		+	(+)
Double drum-dried milk				+	+	+	(+)		+	(+)		+	(+)
Baby food products:													
Semper Välling				+	+	+	(+)		+	(+)		(+)	(+)
Baby Semp				+	+	+	+		+	(+)		+	+
Allergilac				+	+	+			+				

antigenic after heating to 100 C for at least 15 minutes, whereas the major immune globulin component as well as some other proteins were destroyed by heating to 50–60 C for the same length of time (Tables 1 and 2). When judging the results obtained it should be noted, however, that it is not clear how the destruction of antigenicity is related to the physico-chemical changes of the proteins. Furthermore the technique employed is very sensitive and thus minimal amounts of antigenic material can be demonstrated and visible precipitation lines may be formed by proteins present in very low quantity. Thus, if only a small amount of a substance has remained unchanged after treatment, it may still give rise to a precipitation line. It is unlikely that this condition should not be observed, as in such a case the line should form further from the immune serum basin owing to the reduced quantity of antigen. Sometimes the line will appear fuzzy and out of balance since optimal proportions may not be attained. Preparations heated at somewhat lower temperatures than the one at which antigenicity of the protein was destroyed often contained the protein with such a reactivity that a fuzzy and ill defined precipitate was formed (indicated by (+) in Tables 1 and 2).

Comparison of the results obtained with colostrum and mature milk showed that the heat-sensitivity of some proteins was different in the two fluids (cf. line 5, serum albumin, and line 6 in Tables 1 and 2). This may be explained by the afore-

mentioned difference in milieu giving rise to such differences in the heat-sensitivity (9). Several other substances showed similar reactivity. The heat-stability of casein in both colostrum and mature milk was especially notable as the casein precipitates remained quite unchanged after heating the fluids to 120° for 15 minutes.

The immune electrophoretic patterns indicated that the heat effect of the pasteurization at 64°C for 30 minutes was less drastic t the milk proteins than pasteurizations at 5°C for 10 seconds and t 90°C for 120 seconds. In the same way it was found that the proteins of spray-dried milk were somewhat less affected than those of drum-dried milk.

It was possible to analyze milk proteins in food products with the immune electrophoretic technique. These products were made of pasteurized pre-heated and spray-dried milk and the findings agreed with the changes that were obtained after heating of milk to corresponding temperatures. Immune electrophoresis may be a method of controlling the existence of milk proteins in different products and of determining the degree of denaturation f these proteins. It has recently been found that falsification of bovine blood plasma by addition of casein can easily be detected using anti milk sera (").

A sample of a milk product reported to be suit ble for infant hypersen itiv to milk (Allergilac) wa also analyzed. It was found that the α lact lbumin β lactoglobulin and case had retained their antigenicity. These proteins have been demonstrated to be llergens (13 and others). It m be mportant t realize however that the antigenicity of a substance as shown with antibodies from a hyperimmunized animal is not necessarily indicative of the allergenic properties of the substance. The use of such hyperimmune sera to analyze allergens by diffusion in-gel methods ha been criticized from thi point of view (1). It should be mentioned, though that antibodies against bovine milk have been demonstrated by diffusion-in-gel methods in some sera from children hypersensitive to milk (") and it is possible that comparative analyses of these human sera and the hyperimmune sera might give more information about the antibodies detectable in human sera and the allergenicity of bovine milk proteins.

Summary

With immune electrophoretic analysi a descriptive and illustrative picture is obtained of the changes of the milk protein due to heat. It is apparent that casein resists a temperature of 120 C and that α lactalbumin and β-lactoglobulin are still antigenic after heating to 100 C for at least 15 minutes whereas the major immune globulin component as well a a few other proteins are destroyed after heating to 70–80°C for 15 minutes (T bles 1 and 2). The investigation con ern colostrum mature milk milk pasteurized by various method dried milk some commercial bal y food pr lucts and a sample of a milk product advertised a non-allergenic. The possible application of the reported result t the tudy of cow milk llergies discussed

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References

1 Augustin, R.: Pollen antigens versus pollen allergens. Limiting aspects of gel diffusion methods for assay purposes in allergy. *Int Arch Allergy 11* 18–222 1957

2. Ewerbeck, H. Zum Problem der Kindermilch im Licht der modernen Milchwissenschaft. *Milchwissenschaft, 7* 369–375, 1952.

3. Grabar, P. and Williams, C. A. Méthode permettant l'étude conjuguée des propriétés électrophorétiques et immunochimiques d'un mélange de protéines. Application au sérum sanguin. *Biochim Biophys Acta 10* 193–194 1953

4 Grabar, P. and Williams, C. A.: Méthode immuno-électrophorétique d'analyse de mélanges de substances antigéniques. *Biochim Biophys Acta 17* 67–74 1955.

5. *Gugler, E., Bein M. and Muralt G. von* Über immunoelektrophoretische Untersuchungen an Kuhmilchproteinen. *Schweiz Med Wschr 89* 1172 1173, 1959

6. Hanson L. Å.: Immunological analysis of bovine blood serum and milk. *Experientia 15* 471–472, 1959

7 — Unpublished results.

8. Hanson L. Å. and Johansson B.: Immune electrophoretic analyses of bovine milk and purified bovine milk protein fractions. *Experientia 15* 377–379 1959

9 Kastl, H. Veränderungen der Kuhmilchproteine unter Hitzeeinwirkung I Physiko-chemische Veränderungen beim Pasteurisieren, Kochen und bei der Herstellung evaporierter Milch. *Z Kinderheilk 60* 530–538, 1938.

10 Kleinschmidt H.: Über Milchanaphylaxie *Msch Kinderheilk 11* Orig 644–652, 1912.

11 Moro, E.: Biologische Beziehungen zwischen Milch und Serum. *Wien Kli Wschr 14* 1073–1077 1901.

12 Ratner, B. and Gruehl, H. L.: Anaphylactogenic properties of milk. Immunochemistry of the purified proteins and antigenic changes resulting from heat and acidification. *Amer J Dis Child, 49* 287–306, 1935.

13 Ratner, B., Dworetzky M., Oguri, S. and Aschheim, L. Studies on the allergenicity of cow milk. I. The allergenic properties of alpha-casein, beta-lactoglobulin and alpha lactalbumin. *Pediatrics 22* 448–452, 1958.

14 Schulze, K. E. and Müller, F Elektrophoretische Studien an Milchproteinen. Die Untersuchungen von pasteurisierten Milchen und Milchprodukten mit Hilf der Papierelektrophorese. *Milchwissenschaft, 10* 270–274 1955

15. Wadsworth, C. and Hanson, L. Å.: Comparative analysis of immune electrophoretic precipitates employing modified immune electrophoretic technique *Int Arch Allergy 17* 165–177 1960.

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ACTH Induced Thymic Atrophy

by STIG-IVAR BJÖRKLUND

In roentgen examination of the thorax in children one occasionally finds either a diffuse, poorly delineated widening of the mediastinum so that the contours of the heart and big vessels cannot be definitely determined or a fairly well circumscribed tissue mass, the nature of which is sometimes obscure. Most often this mediastinal widening whether diffuse or more distinct is composed of thymic tissue. Indeed, this mediastinal image may have such an appearance that a tumor or malformation cannot be excluded. The size and position of the thymus varies considerably in children.

In order to determine the nature of the mediastinal widening and to increase the diagnostic acumen regarding the mediastinum one can make use of the thymolytic effect of adrenocorticosteroids. Soffer *et al* (2) administered ACTH to 5 patients with an enlarged thymus and in all obtained a shrinkage of the thymus. Caffey & di Liberti (1) have recently reported a similar investigation. In 7 of 8 cases in which idiopathic widening of the mediastinum was discovered by chance in random roentgenograms, the oral administration of adrenocorticosteroids caused rapid shrinkage of the mediastinal image. In

TABLE 1 *Thirteen cases of mediastinal widening treated with ACTH*

Case no.	Age in months	Acton Prolongatum I U daily dose	Acton Prolongatum I U total dose	Thymolytic effect	Remarks
1	6	6	12	0	Regular ACTH
2	3	10	70	+	
3	3	10	60	+	3 weeks later thymic regrowth
4	5	10	60	+	2 weeks later thymic regrowth
5	3	10	30	+	
6	8	10	70	+	
7	36	15	60	0	Ganglioneuroma
8	2	12.5	5	+	
9	1	8	45	+	
10	18	18	5	+	
11		10	60	+	
12	2	10	60	+	
13	2	8	40	+	



Fig 1 Case Not the tumorous mass of the upper right part of the mediastinum.

the eighth case the widened mediastinal image remained unchanged after teroid treatment

Since 19.. we have administered ACTH to hospitalized patient with widening of the media tinum of obscure etiology in order t achiev hrinkage of a large th mic tissue The cases are presented in Table 1 and Figs 1

Results and Discussion

In 11 of the 13 cases the mediastinal widening which was discovered by chance during roentgen examinations, decreased after the administration of ACTH We have interpreted this shrinkage as due to atrophy of the thymus on ACTH administration In Case 1 ACTH did not have any



Fig 2. Case After administra ion of ACTH the widening shown in Fig 1 has disappeared.



Fig. 3 Case 4. "Widening of the mediastinum" does not permit diagnosis of the heart or big vessels.

effect. The mediastinal widening can, however have been composed of thymi tissue because the patient received, according to our present understanding entirely too small a dose of ACTH for entirely too short a period In Case 7 the sharpl delimited formation did not decrease at all, the subsequent operation disclosed that it was composed of a ganglioneuroma. None of the patients exhibited any undesirable reactions.

In Cases 3 and 4 renewed roentgen examination revealed that the decrease in the width of the mediastinum which was observed during ACTH administration had again increased, three and two weeks

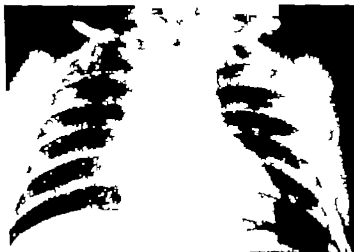


Fig. 4 Case 4 After administration of ACTH the mediastinal widening has disappeared.



Fig. 5. Case 5. Note the "mediastinal widening.

later respectively Thus, with the short course of ACTH therapy described above one cannot expect permanent reduction of a large thymus.

Case 7 demonstrated that administration of ACTH affords a possibility of a differential diagnosis between thymic tissue in the mediastinum and other tumorigenic tissue there Thymic tumors i e. thymoma, seem to respond in the same manner as normal thymic tissue In the material presented by Soffer et al. "in 2 instances the thymic enlargement was proven to be due to tumor 1 of which was malignant in character with proven metastases These thymic enlargements decreased on ACTH administration.

Caffey & di Liberti s patients received adrenocorticosteroids or derivatives thereof orally (prednisone in most cases triamcinolone in a few) The advantage of the administration of these agents over ACTH



Fig. 6. Case 5. After administration of ACTH the widening of the mediastinum has disappeared.



Fig 7 A rounded tumor formation is seen pically on the right side Administration of ACTH did not change the size or ppearance of this formation. Ganglioneuroma.

is that they can be given to ambulatory patients. In Case 1 we used regular ACTH in the others, acton prolongatum. By using the depot preparation it is possible to reduce the number of injections to one per day The disadvantage of injection is counterbalanced by the belief that it is more physiologic to stimulate the adrenal cortex with ACTH than to depress its function with adrenocorticosteroids. However either should be of pproximately the same value as far as the desired effect on the thymic tissue is concerned

Reduction of mediastinal widening during administration of ACTH or adrenocorticosteroids does not always necessarily indicate that it has been thymic tissue or a thymic tumor which has decreased, other tumorous masses may perhaps be affected in a similar manner by ACTH or adrenocorticosteroids In our cases with the exception of Case 7 there were no indications in the clinical picture or the subsequent course that the mediastinal widening should have been composed of other benign or malignant tumors. In Case 1 where we evidently administered too small a dose of ACTH the patient on examination 7 years later was healthy and roentgenography of the thorax at that time did not disclose any widening of the mediastinum

Summary

In 15 cases with a widening of the mediastinum, discovered in the course of roentgenologic xamination, ACTH was administered to increase the possibilities of diagnosing the cause In 11 cases there was a reduction of the width of the mediastinum. The decrease is considered to be due to the thymolytic effect of the adrenocorticosteroids. In one case the widening remained unchanged, in thi case entirely too small a dose of ACTH was given. In another case the more circumscribed enlargement remained unchanged and the subsequent operation revealed that it wa composed of a ganglioneuroma

References

1 Caffey J and di Liberti, C: Acute atrophy of the thymus induced by adrenocorticosteroids: observed roentgenographically in living human infants *Amer J Roentgenol 32* 530 1959

2 Soffer, L. J Gabrilove, J L. and Wolf R. R.: Effect of ACTH on thymic masses. *J Cl Endocr 12* 600 1952.

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Catecholamine Excretion in the Newborn Period Effect of Short Periods of Induced Hypoxia[1]

by LEO STERN[2] ROBERT E. GREENBERG[3] and JOHN LIND

The response of the newborn to induced anoxia is a many faceted one Cross *et al* (5 6) reported that a reduction in the oxygen content in the inspired air from 20% to 15% caused a fall in O consumption in newborn term and premature infants. Cross and co-workers have further shown that, in response to the administration of 15% O_2, premature and full term infants show only a transitory hyperventilatory response which returns to its initial level after approximately two minutes (7) Rowe & James produced changes in pulmonary arterial pressure as well as a fall in the arterial oxygen saturation in a new born infant by the administration of a gas mixture containing 15% oxygen, though the effects were more striking when the oxygen content was lowered to 10% (21)

Asphyxia produced by tracheal compression, has been found to result in intense activation of the adrenal medulla in animals. Rapela & Houssay observed a striking increase in the secretion of the adrenal medulla of the dog in asphyxia (20) similar findings were recorded in the cat by Euler & Folkow (12) In both studies the percentage of noradrenaline in the suprarenal venous blood was similar to the resting state.

It has also been suggested by Dawes and co-workers that the release of catecholamines in response to asphyxia provides an alternative method of closure of the ductus arteriosus in newborn lambs (9) (Paradoxically the other stimulus to closure is an increase in oxygen tension.) Clinically Engleson, Rooth & Sjöstedt have recently proposed the routine use of 15% oxygen as a milieu for premature infants on the supposition that such a mixture is more suited physiologically to their metabolic requirements (11)

The following study was undertaken in order to evaluate the possible relationships between the administration of a reduced oxygen mixture and the excretion of noradrenaline and adrenaline in the newborn infant. Coincident with the above, the study afforded an opportunity to assess normal dopamine excretion in the newborn period.

Methods

Twenty-one full term male infants, ranging in age from 1 t 18 days, were studied.

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The infants were adjudged mature by birth weight (> 2500 g) and/or clinical appearance. At the time of study they ranged in weight from 2300 g to 4480 g. In addition, the study was carried out on four premature infants weighing between 1690 and 2070 g.

Urine was collected according to previously described methods (18). Immediately following collection of the control specimen the infant was placed in a special hood used clinically for purposes of antibiotic inhalation therapy. The inlet was attached to a tank containing a commercially prepared mixture of 84.5% nitrogen and 15.5% oxygen (AGA); the infant was exposed to this mixture for 15 minutes. The oxygen concentration, in front of the infant's airway was repeatedly checked during the period of administration with a Beckman D oxygen analyzer accurately adjusted to read 100% in pure oxygen and 20.9% in room air. An oxygen concentration of less than 16% was reached within 1 minute after the administration of the gas mixture was begun in all infants studied. Respiratory rates were recorded before, during and at the conclusion of the test period. The next observed voiding was collected and the time noted. Two to three drops of 6N HCl were added to the urine specimens and they were immediately frozen; analysis for catecholamines was done usually within 24 hours.

Urinary noradrenaline and adrenaline were determined according to the method of Euler & Lishajko (13). Fluorescence was read in a Coleman photofluorometer Model 12C, with appropriate filters. Dopamine was determined according to the fluorimetric method of Carlsson & Waldeck (2) after adsorption on an alumina column and elution with 0.25 N acetic acid. An Aminco-Bowman spectrophotofluorometer was used for these determinations. It was confirmed that noradrenaline, adrenaline, normetanephrine, metanephrine and 3,4-dihydroxyphenyl acetic acid (dopac) did not produce significant fluorescence under the conditions of the method used. All values were calculated and expressed as nanog per kg body weight per minute (1 nanog = 0.001 microg).

Results

The Effect of a Reduced Oxygen Atmosphere on the Excretion of Noradrenaline and Adrenaline

Clinically, no adverse effects were noted as a result of the administration of the gas mixture used. Respiratory rates remained unchanged in all infants. Rectal temperatures in the full term infants ranged from 35.0 to 37 C. The results for noradrenaline excretion are shown diagrammatically in Fig. 1 and for adrenaline excretion in Fig. 2. The mean values for noradrenaline excretion pre- and post-stimulus are 0.301 ± 0.183 and 0.340 ± 0.177 nanog/kg/minute, respectively. Similar values for adrenaline excretion are 0.056 ± 0.04 and 0.085 ± 0.054 nanog/kg/minute, respectively.

From the above, it is apparent that there is no significant difference between the pre- and poststimulus levels for either noradrenaline or adrenaline when the infants are evaluated as a group. Age had no apparent influence on adrenaline excretion. However, whereas only 1 of 10 infants under 5 days of age showed an increase in noradrenaline excretion following exposure to the gas mixture, 7 of 11 infants over 5 days of age demonstrated an increase of 20% or more in the poststimulus period. Fig. 3 shows this relationship between increasing age and the cumulative percentage of infants who responded to the stimulus with an increase in urinary excretion for both noradrenaline and adrenaline. None of the four prematures studied showed an increase in either noradrenaline or adrenaline (Fig. 4).

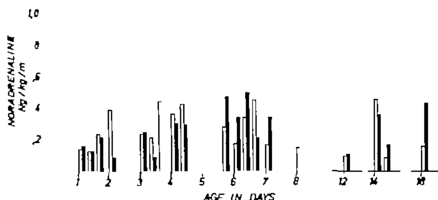


Fig 1 Urinary excretion of noradrenaline in response t 15.5 % O_2 at different postnatal ages. The clear blocks indicate pre-stimulus levels, while the dark blocks indicate the corresponding poststimulus values. Expressed as nanog excreted/kg body weight/minute.

The Excretion of Dopamine in the Newborn Period

No positive correlation between dopamine excretion and the hypoxic stimulus was found. To assess the mean values, then, for dopamine excretion, all values obtained for both dopamine and noradrenaline on each newborn studied were averaged, with the results shown in Table 1 The mean excretion of dopamine is 2.96 ± 1.59 nanog/kg/minut as compared to 0.314 ± 0.123 nanog/kg/minute for noradrenaline Dopamine, then, is excreted in amounts approximately 0 times that of noradrenaline Fig 5 shows that the level of urinary dopamine increases in a rough correlation with increasing levels of urinary noradrenaline Statistically significant dif

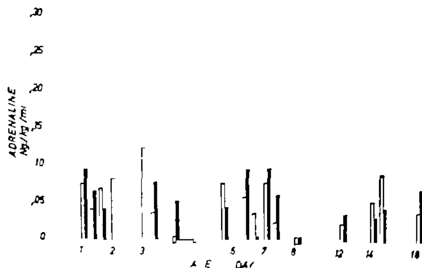


Fig. 2. Urinary excretion of adrenaline in response to 15.5 % O t different p stnatal ages. Legend as for Fig 1

TABLE 1 *The urinary excretion of dopamine (hydroxytyramine) as compared to noradrenaline in normal newborn infants*

Age days	Weight kg	Noradrenaline ng/kg/min	Dopamine
1 1	3.1	15.	1 79
1.5	3.10	131	1 L.
1.7	3.18	.236	3.4
1 9	3.20	.24	1 9
3.3	4.45	138	3.11
3.4	4 1	.353	.46
3.9	2.45	.508	.94
4	2.60	.342	.91
6	.51	418	1.99
6	[illegible]	435	3.16
6	2.82	324	5.65
	3.35	.331	3.41
8	2.45	492	1.69
14	2.63	.225	1.44
18	3.60	402	6.65
	Mean =	.314	2.66
	S.D =	± 123	± 1.49

Since in thi study the mean pre- and post-stimulus values for both noradrenaline and adrenaline are not significantly different when analyzed as an entire group it would appear that either the newborn infant is incapable of responding to this stimulus with an increase in catecholamine excretion or the stimulus itself is not of sufficient severity to produce such a response In either case this would suggest that physiologic responses reported as a result of the administration of hypoxic mixtures of this degree are mediated by mechanisms other than catecholamine release

A more detailed analysis showed that age did not affect the randomness of the response in term of adrenaline excretion. However whereas 7 of 11 nfants over 5 days of age exhibited an increase in noradrenaline excretion of at least 50% after exposure to the gas mixture only 1 of 10 infants under 5 days of age demonstrated such a response. If this finding is valid, it would suggest that either there is a delay in the maturation of the central response to hypoxia (with respect to noradrenaline release) or that the response *to a similar degree of hypoxia is qualitatively different with increasing postnatal age*

The Urinary Excretion of Dopamine

Dopamine was originally demonstrated to be a substance normally found in human urine by *Holtz et al (10) Euler Hamberg & Hellner confirmed its presence in* human urine by chromatographic techniques (14) Drujan *et al* (10) found dopamine in the urine of adults in levels of 108 ± 37 microg per day approximately 5–10 times that of noradrenaline From *our studies, it is apparent that a similar dopamine noradrenaline ratio is present in the urine of newborn infants.*

The physiologic significance of dopamine in tissues or urine has yet to be established It has been demonstrated that dopamine serves as a precursor in the biosynthesis of both noradrenaline and adrenaline in the adrenal gland *(13) and of noradrenaline in sympathetic nervous* tissue (17) Of the entire sequence of reactions in the formation of adrenaline from phenylalanine, the enzyme system that catalyzes the hydroxylation of dopamine to noradrenaline is least understood. Further *Senoh et al (22) have recently shown that dopamine can form* ., 4 5-trihydroxyphenethylamine (hydroxydopamine) both *in vivo* and *in vitro* a compound extremely difficult to separate from noradrenaline by chromatographic techniques; they note that previous studies con-

cerning the biosynthesis of noradrenaline from dopamine must be re-evaluated in the light of these findings. If, as is likely noradrenaline is indeed formed by the hydroxylation of dopamine recent studies suggest that such a precursor role is not the only function of dopamine. In brain, dopamine is present in high concentrations in the caudate nucleus, whereas noradrenaline is most abundant in the hypothalamus (3). Further in ruminants, a high concentration of dopamine is found in many tissues, including lung, duodenum and liver unrelated to other catechol amines; the dopamine content varies with the numbers of a special type of chromaffin cell (1). These studies would suggest that dopamine has a physiologic role of its own in those areas where it is present in high concentration. Dopamine has not, however been detected in either bovine or human plasma under normal conditions, in spite of its presence in both tissues and urine (15 23). It would thus seem that the kidney itself contributes significantly to the relatively high urinary dopamine levels; as such the urinary excretion of dopamine can probably not be used as an index of endogenous extra renal production. The delineation of the functions of dopamine in the organism remains as an important and interesting task.

Summary

It has previously been demonstrated that newborn infant are capable of responding to change of posture nd insu lin hypoglycemia with an increase in catecholamine excretion. Similarly previous studies by other workers have shown that severe anoxia at lea t evokes a marked release of catecholamines in animals. When newborn infants were exposed for 15 minutes to a gas mixture containing 15.5 % oxygen and 84.5 % nitrogen no significant change in the urinary excretion of noradrenaline and adrenaline ensued. This would suggest that either the newborn is incapable of responding to this stimulus with an increase in catecholamine excretion or the stimulus itself is not of sufficient severity to produce such a response. In either case any physiologic response in newborns following exposure to a hypoxic mixture of this degree would then be mediated by mechanisms other than catecholamine release.

The study also suggests, however that increasing postnatal age exhibits a positive correlation with the response to the hypoxic stimulus used, in terms of increases in noradrenaline excretion. The validity of these findings as well as their significance must await confirmatory studies.

Dopamine was found to be present in the urine of newborn infants in amounts roughly nine times that of noradrenaline a ratio parallel to that observed by others for adults. No correlation between the hypoxic stimulus and dopamine excretion was found. The present confusion concerning the significance of dopamine in both urine and tissues, is briefly reviewed.

Acknowledgements

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References

1 Bertler, A., Falck, B Hillarp N-Å Rosengren E. and Torp A.: Dopamine and chromaffin cells. *Acta Physiol Scand, 47* 251 1951
2. Carlsson A and Waldeck, B.: A fluorimetric method for the determination of dopamine (3-hydroxytyramine) *Acta Physiol Scand, 44* 293 1958.
3. Carlsson A.. The occurrence distribution and physiological role of catecholamines in the nervous system. *Pharm Rev 11* (Pt) p 374 1959
4. Celander, O The range of control exercised by the sympathic-adrenal system *Acta Physiol Scand 4* Suppl. 116 1954
5 Cross, K. W Tizard J P M and Trythall, D A. H The metabolism of newborn infant breathing 15 oxygen. *J Physiol 129* 69, 1955.
6. — Gaseous metabolism of the newborn infant breathing 15 % oxygen. *Acta Paediat 47* 17 1958.
7 Cross, K. W Factors ffecting O consumption in newborn infants. In Adapt tion t Extrauterine Life ed. T K Oliver Ross Lab., Columbus, 1960
8. — Personal communication.
9 D wes, G S.: Changes in the irculation at birth nd the effect of asphyxia. Recent Advances in Paediatrics, ed D Gairdner T and A Churchill Ltd London, 1958.
10 De jan B. D Rockers, T L L ven, D S and Murphy G F The differential determination of catecholamines in urine *Canad J Biochem Physiol 37* 1183, 1959.
11 Engleson G Rooth G and Sjöstedt S.. Treatment of premature infant with 15 oxygen *Acta Paediat, 48* Suppl. 118 4 1959
12 Euler, U S and Folkow B Einfluss verschiedener afferenter Nervenreize uf die Zusammensetzung des Nebennierenmarkinkretes bei der Katze *Arch Exp Path & Pharm 219* 242 1953
13. Euler, U S and Lishajko, F The estimation of catecholamines in urine *Acta Physiol Scand, 45* 122, 1959.
14 Euler, U S. Hamberg, U and Hellner, S.: β-(3,4-dihydroxyphenyl) ethylamine (hydroxytyramine) in normal human urine *Biochem J 49* 655 1951
15. Euler, U S v Floding, I. and Lishajko, F The presence of free and conjugated 3:4-dihydroxyphenylacetic acid (Dopac) in urine and blood plasma. *Acta Soc Med Upsal 64* 217 1959.
16. Goodall, McC. and Kirshner, N Biosynthesis of adrenaline and noradrenaline in vitro. *J Biol Chem, 226* 213, 1957
17 — Biosynthesis of epinephrine and norepinephrine by sympathetic nerves and ganglia. *C rculation, 17* 366, 1958.
18. Greenberg, R. E., Lind J and Euler, U S. v Effect of posture and insulin hypoglycemia on catecholamine excretion in the newborn. *Acta Paediat 49* 780, 1960
19. Holtz, P Credner, K. and Koepp W Die enzymatische Entstehung von Oxytyramine in Organismus und die physiologische Bedeutung der Dopadecarboxylase. *Arch Exp Path & Pharm, 200* 356, 194..
20. Rapela, C. E. and Houssay B. A.: Adrenalina y noradrenalina de la sangre suprarenal del perro durante la asfixia. *Rev Soc Argent Biol, 28* 7 1952.
21 Rowe, R. D and J mes, L. S.: Pattern of response of pulmonary and systemic arterial pressures in newborns and older infant t short periods of hypoxia. *J Pediat, 51* 7 1957
22. Senoh, S., Creveling C. R., Udenfriend, S. and Witkop B.: Chemical, enzymatic and metabolic studies on the mechanism of oxidation of dopamine *J Amer Chem Soc, 81* 6236, 1959
23. Weil-Malherbe, H and Bone, A D The fluorimetric estimation of adrenaline and noradrenaline in plasma. *Biochem J 67* 65, 1957

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The Mercury In Rubber Strain Gauge for Measurements of Blood Pressure and Peripheral Circulation in Newborn Infants

by OLOV CELANDER and GÖRAN THUNELL

In previous communications (1 2) it was described how venous occlusion plethysmography could be succesfully performed on newborn infants for measuring changes in leg blood flow peripheral resistance to flow and systemic blood pressures In a further study (3) on medical students the plethysmographic method for blood pressure determinations was found to agree satisfactorily with simultaneous intraarterial pressure recordings from the corresponding artery However in clinical work the use of a water filled plethysmograph and the recording on an ordinary kymograph on smoked paper is rather inconvenient. In this paper will be described how these difficulties might be overcome by the use of the "mercury in rubber" gauge which was introduced in the study of peripheral circulation in man some years ago by Whitney (5).

Methods

Principle

The mercury in rubber gauge operates as fairly distensible electrical strain gauge A suitable length of thin and highly distensible rubber tubing is filled with mercury and fitted to the extremity The electrical resistance of the mercury column is dependent on the circumference of the leg and, therefore, on leg volume An increase of leg volume will distend the gauge and, as the mass of mercury remains constant, the length of the mercury column will increase on behalf of a corresponding decrease of thickness. Both these changes will produce an increase of electrical resistance The gauge is part of a balanced Wheatstone bridge, fed with a low voltage, high frequency current. The bridge, being balanced for a particular degree of extension of the tube, will allow changes of extension (plus or minus) to be recorded by the current flowing in the bridge galvanometer circuit. Whitney (5) has shown that a good linear relationship exists between the bridge output recorded by the galvanometer and the percentage extension or contraction of the gauge To attain maximal sensitivity of the "mercury in-rubber gauge we have (following the recommendations to us by Dr C. J Eagan) made the gauge make up practically the entire resistance of one arm of the bridge. The current feeding the bridge was obtained from an ordinary electromanometer (Elema). The current flowing in the galvanometer circuit of the bridge was amplified in the electromanometer and could be further amplified by the amplifier in the electrocardiograph (Elema Cardirex 3B), which was used for the recording These general principles are illustrated in simplified form in Fig. 1

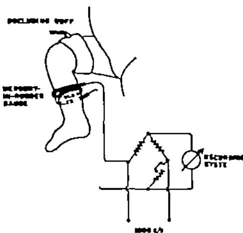


Fig 1 A schematic drawing of the "mercury in rubber" strain gauge and the Wheatstone bridge

The "mercury in rubber' gauge

The rubber tubing used has the transverse dimensions of 1 1 mm (outer diameter) and 0.35 mm (inner diameter) The leads to the bridge are fine insulated multi-strand copper wire The contacts which intrude into the rubber tube have been made of 0 7 mm silver threads, 4 cm of length, soldered up in one end with the copper wire. The other end, which is to be introduced into the tubing is soldered up with two thinner (0 18 mm) silver threads; t facilit t contact with the mercury they protrude bout 1 cm beyond the tip of the thicker silver thread.

The filling of the tube with mercury requires pains and practice. It was found convenient to place the tube level on a firm table. An ordinary 5 cc glass syringe supplied with a thin hypodermic needle which has a dulled tip, was found suit ble The prepared tips of the silver leads were then carefully introduced and the tube ends were sealed by tight ligatures. Some over filling" with mercury was found desirable The gauge was now tested for electrical continuity and for its capacity to withstand rather violent handling

Latex rubber tubing (nr 27), obtained from Huntington Rubber Mills, Box 570, Portland, Oregon, U.S.A.

The transformer coupling device

The circuit diagram is shown in Fig 3. The bridge excitation (A–B) is supplied by the ordinary Elema electromanometer which normally supplies a pressure transducer with an alternating current of 11 V at 1000 cps. T_1 and T_2 are matching transformers. R is a variable resistance choosen to match the resistance of the "mercury in-rubber" gauge (1–2) The current over the bridge (C–D) supplies the electromanometer and is recorded on the electrocardiograph After balancing the bridge for the particular ex tension of the rubber tube, when fitted to the calf of the child, a suitable amplification is choosen on the electromanometer and the electrocardiograph.

The use of the mercury-in-rubber" gauge on newborns

The general principles for plethysmography and the use of this method for blood-pressure determinations ha e been described elsewhere (1 2) T be successful in using the highly sensitive "mercury in rubber" gauge, mechanical disturbances have t be minimal. The child, therefore has to be at complete rest, preferably asleep during the measurements. The blood-pressure cuff the mercury manometer and the pressure reservoir are the same as those previously described by Celander & Thunell (2) Fig 2 shows the apparatus and the gauge fitted to the calf of a newborn.

Results

Fig 4 shows at high amplification, the minute volume changes in the leg of a newborn which accompany the arterial pulse waves. From this tracing it will be easily realized that the electrical strain gauge is well suited as a "pulse indicator when measurement of the systolic arterial blood pressure is desired. As a matter of fact, the amplification of the arterial

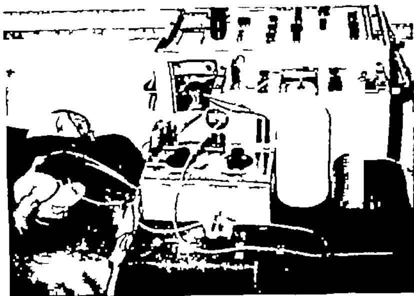


Fig. 2. The "mercury-in-rubber" gauge fitted to the calf of a newborn infant. The pressure reservoir for the cuff, the mercury manometer and the box containing the bridge are shown in front of the electromanometer and the electrocardiograph.

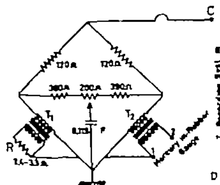


Fig 3

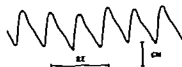


Fig. 4.

Fig 3. Circuit diagram for the transformer coupling device. An Elema electromanometer supplies the 11 volt, 1000 cycles per second bridge excitation at A and B. The bridge output, connected to C and D is amplified by the electromanometer and the recording electrocardiograph. The mercury in rubber gauge is connected to points 1 and 2.

Fig 4. Arterial pulsations in the leg of a newborn recorded by the "mercury-in-rubber" gauge. High amplification. The tracing illustrates the possibilities of the electrical strain gauge as a pulse indicator for measuring systolic blood pressure.

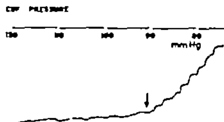


Fig. 5. A determination of the systolic blood pressure in newborn infant. Note the appearence of prominent arterial pulsations and the sudden increase of leg volume once the cuff pressure is released to pressures below 90 mm Hg

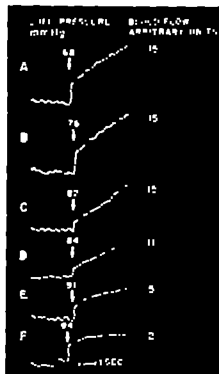


Fig. 6. Measurements of the rate of arterial blood flow in relative units at various cuff pressures during the collecting periods. The systolic pressure must be very close to 94 mm Hg (which is the highest cuff pressure at which minimal arterial inflow was recorded). Similarly diastolic pressure must be very near 82 mm Hg (the highest cuff pressure for maximal arterial inflow). Note at C D E and F that only minimal arterial pulsations remain in the tracings in spite of clearly positive flow records.

pulsations is practically unlimited although in ordinary use a considerably lesser degree of amplification is quite sufficient for this purpose How such a determination of systolic pressure can be achieved is illustrated in Fig 5 The occluding cuff fitted to the thigh was first inflated at a suprasystolic pressure which was then released at a constant speed marked in the upper tracing The tracings allow an accurate determination of the systolic pressure in the artery to the extremity for two reasons. Firstly the appearance of arterial pulsations beyond the occluding cuff at a pressure of 90 mm Hg is good evidence that at this very level the intraarterial systolic peaks superseded the cuff pressure Previous rhythmic variations in the bottom tracing were due to the respiration of the child and were not synchronous with the heart rate Secondly at the same cuff pressure the tracing of the "mercury in-rubber" gauge suddenly rises rather abruptly This sudden increase of leg volume reflects the rushing of blood into the extremity once the cuff pressure is no longer effective in interrupting arterial inflow but still high enough to block the venous outflow from the extremity In this child the two phenomena coincided at a cuff pressure of 90 mm Hg. In others, as will be seen, they did not. Under such circumstances the determination of systolic blood pressure was decided on the criterion of an actual arterial inflow rather than the less reliable presence of arterial pulsations.

Fig 6 and Fig 7 illustrate how the "mercury in-rubber" gauge can be used to measure both systolic and diastolic blood pressure on the basis of blood flow determinations at various cuff pressures, as was recently suggested by Celander &



Fig. 7 Diagram showing the relation between the pressure in the cuff and the rat of arterial inflow (relative units) to the extremity

Thunell ( ) In the experiment referred to in Fig 6 the highest cuff pressure at which a minimal arterial inflow could be recorded was 94 mm Hg whereas the highest cuff pressure giving a maximal rate of arterial inflow was 8? mm Hg These two values are representative of systolic and diastolic blood pressure respectively In the diagram in Fig. 7 the total number of observations on blood flow at this occasion is plotted against cuff pressure From this diagram it is apparent that both systolic and diastolic blood pressure can be determined with a high degree of accuracy by this approach.

Discussion

The 'mercury in-rubber" strain gauge was originally suggested by Glaser in 1939 (4) to study respiratory moments. After it was adopted by Whitney in 1953 (6) in the study of peripheral circulation in man it has been successfully used in a great number of circulatory studies, when ordinary plethysmographs are difficult to handle In this study the "mercury-in rubber" gauge has been used to determine with great precision at which cuff pressure arterial pulse waves pass beyond the occluding blood pressure cuff. The practically unlimited possibilities of amplification give the electrical strain gauge advantage as a pulse indicator when arterial pulsations are small as is the case in new borns nd in patients with occlusive arterial disease Of probably greater importance is the fact that the electrical strain gauge also clearly shows at which pressure (upon gradual lowering of the cuff pressure) an arterial inflow to the extremity starts. The fact that arterial pulsations might be absent or small in spit of positive flow records (see, e.g Fig 6) has previously been commented upon (2) and gives further status to the electrical strain gauge plethysmograph when compared with other sphygmomanometric methods

Venous occlusion plethysmography of the foot and calf can be used on newborns for measuring not only the systolic but also the diastolic systemic blood pressure (2) This new principle for indirect blood pressure determinations has been checked against the intraarterial blood pressure in adults (3) The "mercury in-rubber gauge has not yet been similarly compared with direct intravascular pressure recordings, but there seems to be no reason why the



Fig. 1. Soft tissue radiograph showing tumour arising from posterior wall of nasopharynx.

had fallen to 12.2 g. % but the leucocytes remained entirely normal. X-rays of the chest and skull showed no change but a view taken to show soft tissues demonstrated a mass encroaching on the posterior wall of the pharynx (Fig. 1). It was thought possible that the child might have a retro-pharyngeal sarcoma or secondary deposits from a neuroblastoma.

An examination under anaesthesia carried out by Mr. A. W. Halfhide revealed a fixed mass in the left naso-pharyngeal area which extended anteriorly to the middle of the nasal fossa and posteriorly to the free border of the palate. A biopsy was obtained which confirmed the presence of a malignant tumour but its nature remained uncertain.

During the next two weeks the proptosis increased. His nose became blocked on the left side and oedema of the left side of the face developed. Signs of pneumonia appeared at the base of the right lung and he died the following day just two months after the original onset of blindness.

Autopsy report

External appearances

The body was that of a slight, wasted boy 108 cm in length and 15.. kg in weight showing generalised pallor. The chief abnormalities affected the head. There was a slight ill-defined subcutaneous eminence over the supra nasal region, together with marked hypertelorism, the nasal bridge being broad and flattened and the interocular distance 4 cm. Gross bilateral exophthalmos was present with some divergence of the ocular axes and severe chemosis. The periorbital tissues were thickened, the face broad and puffy and there was some circumocular ecchymosis. In the buccal cavity the lateral portions of both upper alveolar margins were considerably swollen and encroached on the hard palate. There was gross dissolution of the maxilla, the teeth embedded in the soft tissue were quite loose. In addition there was enlargement of the lower alveolar margins, with considerable mobility of the teeth and gingival bleeding.

Internal appearances

Primary tumour. The nasopharyngeal lumen was reduced to a slit-like cavity by sheath of submucous tumour tissue most massive posteriorly where it attained a depth of 2 cm (Fig. 2). Inferiorly the tumour extended into the palate which was considerably thickened and somewhat nodular on its posterior aspect. Superiorly the growth protruded into both nasal cavities, whilst laterally it had largely destroyed the medial orbital walls and facial bones fungating into the retro-ocular tissues and the maxillary antra. In general, the tumour was composed of pale, cream-coloured tissue of rather soft consistency but in the posterior pharyngeal region it was partly necrotic and deeply discoloured by haemorrhage. In the nasopharynx and nasal cavities the mucosal aspect of the tumour had a distinctly lobulated surface.

Skull. Whilst the basilar portion of the occipital bone appeared intact, the primary



Fig 2. Hemisected specimen, showing the nasopharyngeal tumour in situ

tumour had invaded and largely destroyed the body of the sphenoid and the cribriform plate, extending over the inner aspect to form large plaque which reached a maximum depth of 1 cm. The pituitary fossa was completely destroyed and the pituitary gland submerged in the mass of tissue. The bones of the calvarium were intact but over most of their inner aspect a thin sheet of neoplastic tissue was present deep to the fibrous layer of the dura, and in the medial frontal region externally a subperiosteal plaque of similar nature was present, continuous with that in the orbits. The coronal suture was unduly mobile, the suture line being replaced by a ridge of tumour wich projected on either side

Microscopy

The tumour tissue in all sites showed the typical histological appearances of lymphosarcoma, the type cell being the lymphoblast

From the primary nasopharyngeal tumour there was direct invasion and destruction of the basisphenoid bone and extension as a thick layer beneath the dura. The sella turcica was largely destroyed and only the anterior lobe of the pituitary gland could be identified, it was of small size and embedded within a dense mass of tumour

Multiple sections of the brain revealed diffuse lymphoblastic infiltration of the subarachnoid space and also of many of the perivascular spaces in the subjacent brain substance Similar infiltration was present in the meninges of the lumbar spinal cord and also in the fascicles of the nerve roots.

Problem of diagnosis

At the outset this child s blindness was thought to be due to a retro-bulbar neuritis which is frequently bilateral in childhood, unlike the commoner condition in adults. It is one of the rarer sequelae of measles or may occur following a period of vague ill-health, there is a sudden loss f vision ccompanied by blurring or frank swelling of both optic discs Usually there are no other abnormal neurological signs and complete recovery is the rule within a few weeks (5). Occasionally a bilateral optic neuritis may be the presenting factor in neuromyelitis optica, but it is then commonly accompanied by signs of myelitis.

Doggart (2) makes the point that when visual failure in childhood is of neurological significance there are usually other symptoms to suggest that the trouble is not entirely ocular In this child we were unable to find any other signs during the first three weeks of his illness. In retrospect the enlargement of the left cervical lymph nodes should perhaps have suggested the presence of generalized disease.

Loss of visual function may precede the ophthalmoscopic changes and this is commonly the case when there is compres-

sion of the optic nerve or chiasma by a tumour In childhood such a tumour is commonly a "glioma" of the optic nerve or a craniopharyngioma In the former there is usually unilateral loss of vision accompanied by proptosis and even when the chiasma is involved, it is usual for one eye to be more grossly affected than the other Radiography will show enlargement of the optic foramen. Craniopharyngiomata produce a more gradual loss of vision especially affecting the temporal fields the fundi may show papilloedema or optic atrophy but the loss of vision is often greater than would be expected from the ophthalmoscopic appearances Radiographic changes include destruction of the sella together with calcification above it and the signs of raised intra cranial pressure

Other demyelinating diseases or tu mours affecting the optic radiations or visual cortex may rarely cause blindness but it must be exceedingly uncommon for this to happen without other neurological signs

It was not until this boy developed progressive enlargement of the cervical glands, together with downward displacement of the soft palate that the true nature of the disease was suspected X-ray examination of the skull then showed a soft tissue mass in the nasopharynx and a biopsy confirmed the presence of a malignant tumour

Tumours of the naso-pharynx

Whilst local symptoms such as nasal discharge bloodstained sputum discom fort in swallowing nasal quality of voice and deafness (from Eustachian tube obstruction) often result from naso-pharyngeal tumours they are by no means the most frequent initial manifestations. In a series of 454 cases (7 of whom were children) Godtfredsen (3) found that the commonest initial symptom of malignant tumours arising in the naso-pharynx was cervical glandular enlargement (32%) Next in descending order of frequency were nasal obstruction, with or without excessive nasal discharge (30%) and impairment of hearing, sometimes accom panied by other symptoms of Eustachian canal obstruction (22%) *In 18% of* cases, various neurological syndromes were the mode of presentation, the most frequent of which were trigeminal neural gia or paresis of the sixth cranial nerve Unlike the commoner initial symptoms, which were usually an isolated finding over half of those patients with neurological symptoms had concurrent cervical lymphadenopathy or nasal obstruction. Godtfredsen describes only one case in which vision was affected, a man of 31 who when seen because of nasal obstruc tion, was found to have nystagmus and *impaired vision. In no instance was blind* ness a presenting symptom.

In a group of 132 patients with post-nasal tumour Lambert (4) found that the chief presenting features were cervical metastases (46) nasal obstruction (23) neuralgia (?6) and epistaxis (15) Less frequently the onset was marked by nasal discharge (6), dysphagia (3) proptosis (2) and involvement of the oculo-motor ner ves (2) Again in no instance was blindness a presenting symptom though it occurred once at a later stage of the disease

A case showing many features similar to our own was discussed at the Royal Society of Medicine by Vulliamy (5) The

patient was a four year-old boy who developed unilateral blindness with exophthalmos, and was subsequently found to have a lymphosarcoma arising in the nasopharynx.

Lymphosarcoma in childhood

Though by no means rare, lymphosarcoma must be ranked among the less common neoplasms of childhood. There is marked preponderance of affected males, as at other ages and with other lymphoid tumours, but no distinctive age distribution.

There is no absolute demarcation between lymphosarcoma on the one hand and lymphatic leukaemia on the other and this relationship is emphasised by the relatively frequent occurrence of a leukaemic phase during the course of the former

Lymphosarcoma is one of the most invasive of tumours and its aggressiveness is emphasised by a very rapid evolution in the majority of instances. Apart from actual metastatic dissemination, there is evidence to suggest that the neoplastic process may be multicentric or systematised in its origin. In view of these features any attempt at delineation of the sites of "primary" growths is to some extent arbitrary nevertheless, information on this point has a certain clinical value, in differential diagnosis and lsewhere

Children with lymphosarcoma usually come under medical ttention in one of three ways: with respiratory and/or vascular disturbances from a mediastinal tumour with an abdominal mass which may be retroperitoneal or alternatively involves the bowel wall and frequently leads to intussusception, or with enlargement of a group of superficial lymph nodes, usually cervical in situation. Whilst these are the main modes of presentation, occasional cases of lymphosarcoma may come under observation for a variety of reasons, such as enlargement of salivary glands or testes, subcutaneous masses etc. (1) In the instance described the primary tumour occupied an uncommon situation and gave rise to most unusual manifestations

Summary

1 An unusual case is described of a boy with lymphosarcoma of the nasopharynx who presented with blindness.

_ The problems of diagnosis are discussed.

3 The features of nasopharyngeal tumours and of lymphosarcoma in child hood are briefly reviewed.

Acknowledgements

It is a pleasure to acknowledge our gratitude to Dr Paul Sandifer and Professor A. A. Moncrieff for permission to publish this case W are indebted to Mr Derek Martin for the illustrations.

References

1. Dargeon, H. W.: Lymphosarcoma in childhood. *Advances Pediat* 6 13, Year Book Publishers Inc., 1953.
2. Doggart, J. H.: The neuro-ophthalmological aspects of failure of vision in childhood. *Proc Roy Soc Med*, 47 489 1954
3. Godtfredson, E.: Sarcoma of nasopharynx. *Acta Otolaryng (Stockh)*, *S ppl.*, 59 1944.
4. Lambert, V.: Treatment of carcinoma of nasopharynx. *Proc Roy Soc Med*, 47 547 1954
5. Meadows, S. P.: Failure of vision in childhood. *Proc Roy Soc Med*, 47 497 1954.
6. Vulliamy, D. G.: A case of nasopharyngeal sarcoma. *Proc Roy Soc Med* 41 452, 1948.

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CASE REPORT

Salmonella Septicemia and Aplastic Crisis in a Patient with Sickle Cell Anemia

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The erythroblastopenic or aplastic crisis occurring either in patients with an established hemolytic process or in hematologically normal persons is a *well-documented* syndrome (3 4 5 6 9 10 13) Vague illnesses, suggestive of respiratory infections, proven infectious agents, drugs and toxic agents have been *shown to trigger* its onset

It is the purpose of this communication to report a case of aplastic crisis in a patient with sickle-cell anemia during the course of a septicemia due to *Salmonella typhimurium*

Case Report

O.X. a 4-year-old boy known t ha had sickle-cell anemia since the age of 30 months, was admitted to the hospital h complaints of low grade fever generalized malaise and anorexia of two weeks duration. The patient 13-month old sister remained well.

The past history revealed that the patient had had pertussis t the age of 3 ears H experienced joint pains for the first time t the age of 30 months and he diagnosis of sickle-cell anemia was established H as never transfused nor as he observed t be jaundiced at an time

Physical examination revealed ll developed child who ppeared moderat l ill. Temperature 38 C pulse 120 respirations 32. The sclerae were slightly teric and the mucus membranes pale. Examination of the chest revealed no abnormalities, except for a soft apical systolic murmur. The liver was palpable cm below the right costal margin and the spleen ½ cm below the left costal margin. N joint changes or neurological abnormalities were present

Laboratory data. The urine was normal except for 1 + albuminuria. The hemoglobin concentration was 5.3 g 100 ml. The blood contained 8000 wbc/mm³ with a differential count of 48% nonsegmented neutrophils, 7% segmented neutrophils, 4 % eosinophils, % monocytes and 39% lymphocytes. A sickle preparation was positive. Fetal hemoglobin was 13 %. Before transfusion paper electrophoresis of hemoglobin revealed only S type hemoglobin. Total serum bilirubin was 1.5 mg 100 ml with direct 0.0 and indirect 1.5 mg 100 ml. A direct Coombs test was negat e Chest film was reported as normal

The patient was thought to ha a painful hemolytic crisis. However the reticulocytes ere not counted, nor was special ttention paid to the peripheral smear regarding the degree of polychromasia.

Course On the day of admission the patient was gi en 100 ml of fresh blood, seda t es and aspirin. The hemoglobin level on the next da was 6.8 g 100 ml. F om the second hospital day he was given 30 mg prednisone dail For the first four da of his hospitalization he ran low-grade fever and was complaining of bdominal and joint pains Thereafter he st rted spiking a high fever his jaundice increased and the bdominal pain became more ntense His spleen ceased t be pal-

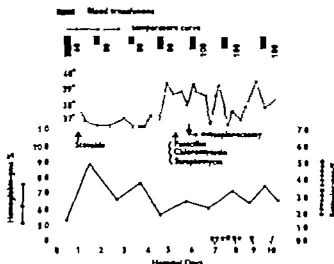


Fig 1 Graphic representation of the clinical and hematological course

pable however the liver was constantly enlarging nd became extremely painful on palpation. A blood culture was btained following which treatment with penicillin, streptomycin and chloramphenicol was begun. On the 7th hospital day the total bilirubin was 7.8 mg/100 ml (direct 5.1 and indirect 2.7 mg/100 ml) The urine showed a 3 + reaction for urobilinogen. Other studies included cephalin floculation 3 + and thymol turbidity 1 +; peripheral blood contained 3000 wbc mm³ with a differential f 12% segmented neutrophils, 23 % nonsegmented neutrophils, 2% eosinophils, 6% monocytes and 57% lymphocytes. The reticulocyte count was 0 0% and the peripheral blood smear contained numerous target cells and sickle cells; one nucleated red blood cell per 100 white blood cells was counted; and thrombocytes were decreased. The marrow was cellular but only few orthochromatophilic normoblasts were seen. The granulocytic and megacaryocytic series showed a shift to the left and the E G ratio was 1 15. A moderate increase in plasma and reticulum cells was present. No giant pronormoblasts were seen.

During the next three days the general condition of the patient rapidly deteriorated High spiking fever continued as did severe bdominal pain mostly in the right upper quadrant The liver was palpable 8 cm below the right costal margin and extremely painful. Digitalization had no effect upon the liver size. At no time during the clinical course did the patient appear to have heart failure, nor were abnormal focal neurological findings detected. On the 11th hospital day the patient became disoriented and expired late in the afternoon of the same day Throughout the clinical course the hemoglobin level was maintained abo 5.8 g/100 ml by fresh blood transfusions. At no time was he given more than 500 ml of blood daily It was of interest to bserve that the hemoglobin level on the day after transfusions fell again to pre-transfusion levels (Fig 1)

Salmonella typhimuri m was cultured from the blood which was btained on the fifth hospital day but wa not reported until two days after the patient death.

Discussion

In some way salmonellosis seems to be specifically related to sickle-cell anemia Of the patients with sickle-cell anemia studied at John Hopkins Hospital the great majority of bacteremias were due to

salmonella organisms (1) In 1951 Hodges & Holt (7) pointed out the correlation between salmonella osteomyelitis and sickle-cell anemia. Since then, there have been an increasing number of reports pointing out this correlation not only for patients with sickle-cell disease but other hemoglobinopathies as well (14) To explain this occurrence several factors have been mentioned, capillary thrombosis in the gastrointestinal tract may predispose to invasion by the intestinal organisms. Debility and "autosplenectomy"[1] in these patients may decrease their resistance to infection, once in the blood stream salmonella organisms may lodge in necrotic and ischemic areas resulting from the sickling process decreased local resistance due to ischemia may permit the growth of dormant organisms, thus explaining the observation that sickle-cell crisis often precedes symptoms of osteomyelitis (17)

Because serial reticulocyte counts and bone marrow studies have not been reported in these particular patients, the frequency of aplastic crisis remains unknown.

Chernoff & Josephson (3) reported the first case of aplastic crisis in a patient with sickle-cell anemia and salmonella cholerae suis bacteremia. A possibl second case was reported by Silver *et al* (14) In retrospect our case is of unique interest because of the many factors involved In addition to the previously described course it appears that in the middle of the septic course, an autosplenectomy took place, the liver increased in size and the patient became more jaundiced

[1] Due to fibrosis, the spleen of patients with sickle cell disease is reduced to ti y mass, thus in this respect these patient practically have no spleen ("Autosplenectomy).

The effect of cortisone and similar adrenocortical steroids in the initiation and progress of bacterial infections remain a controversial issue It has been suggested that relatively small doses may increase resistance to infection in animals (11 1) However the vast weight of evidence suggests that cortisone and similar adrenocortical steroid decrease resistance to infection ( ) Although cortisone has been found to be of value as an adjunct in the treatment of typhoid fever (18) it ha not been shown if hormone therapy should be used in other salmonella infection (15) In experimental salmonellosis it has been shown that, when hormones were administered before inoculation of the rat with sal typhimurium, cortisone increased the mortality even though chloramphenicol had been given at the time of infection. The rapid deterioration of the general condition of our patient may be related to the use of steroids coupled with the autosplenectomy His death was mainly attributed to a overwhelming salmonella typhimurium septicemia

In our case steroids did not have a favorable effect upon the evolution of the plastic crisis. Furthermore they did not prevent the emergence of an aplastic crisis in a patient reported by Silver *et l.* (14) Steroids were found to be of no real value in the management of aplastic crises in two patients reported by Mesh *et l* (9) For these reasons as well as the experimental studies the use of steroid m y be contraindicated in patients with sickle-cell anemia during crisis Steroids may alter the host resistance sufficiently to provide a foothold in these particular patients for salmonella septicemia or they

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CASE REPORT

Tuberous Sclerosis in a Mother and Her New Born Son

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Tuberous sclerosis or Bourneville's disease is characterized by the symptom triad of sebaceous adenoma, epilepsy and mental deficiency. Studies of families have shown it to be an inherited condition in which morphologically well-defined tumour-like foci occur in a number of organs; they can often be strikingly demonstrated in the kidneys, heart and retina. The disease is not confined to any particular germ layer, but may affect one or several. The progressive nature of the cerebral lesion is reflected in the increasing degree of mental deficiency and progressive epilepsy; although less characteristic skin lesions have been described, the sebaceous adenomas are regarded as pathognomonic.

The requirement of the above-named triad has prevented the diagnosis of tuberous sclerosis in many atypical cases. However, by follow up of these atypical cases it has been possible to trace a simple dominant character in up to 4 generations in many families (5, 8, 9). No hereditary factor has been demonstrated in most of the cases published in the literature, however, and it is probable that isolated cases do occur.

About 10 % of the cases in the literature concern children under 3 years of age and almost all of these have been diagnosed at necropsy (4). This is surprising since tuberous sclerosis is a congenital condition that usually makes its appearance before the age of 2 years (3, 5). It is explained by the facts that the first sign of illness is commonly an epileptic fit; that a mental defect may be difficult to assess in early childhood; that the initial skin lesions are uncharacteristic with regard to appearance and localization, the sebaceous adenomas not becoming apparent until a later date; and that the full clinical picture develops gradually and progresses slowly with increasing age.

The present paper is devoted to an account of 2 cases of tuberous sclerosis, in a mother and her child. They are interesting because of the direct inheritance which they illustrate and because post-mortem examination of the infant who was only one day old revealed lesions typical of tuberous sclerosis.

Case 1. M.M.B. (Record no 144/60, Dept of Neurology). The patient was a woman of 28 years. Her parents were unmarried, and the family history is therefore uncer-



Fig. 1. Case 1) Sebaceous adenoma in the face b) Paraungual fibroma and nail changes.) Gingival hyperplasia.

Fig. 2. Case 2 Subependymal nodule in the wall of one of the lateral ventricles. Magnification 10

tain. Her father cousin was said t have major epileptic fits. See below regarding the patient son.

The patient had an 8-year elementary school education, after which she had worked as housemaid until she married at 27 years of age.

Past illnesses included infectious hepatitis at the age of 10 years. Since the menarche had not occurred at 17 years, hormone treatment was instituted and continued for 2 years, at which time the periods became regular and apparently normal.

From bout the age of 17 the patient suffered from fits; thus, these made their pearance at about the time hormone treatment was commenced. The patient states the fits start with dizziness and feeling of fainting; she has no memory of what happens during the next 30–60 seconds. Although the ttacks may occur t any time, for example when the patient is drying dishes, when she is out cycling or when tending to a child, she is able to continue her work, the only outward sign being pallor and flushing of the face. Without treatment she had up to about 10 fits per day but following treatment with phenantoin and phenobarbital which was started in 1953 they have occurred only at long intervals. There have never been convulsions during these fits; she has never fallen t the ground, has never been incontinent of urine or faeces, nor has she ever bitten her tongue.

The patient became pregnant in 1959, the dat of her last menstrual period being

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CASE REPORT

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Case I M.M B (Record no 144/60, Dept f Neurology) The patient was a woman of 28 years. Her parent were unmarried, and the family history is therefore uncer



Fig. 2. Microphoto of the myocardial and cerebral changes in Case 2.) Myocardiosis with changed muscle fibres in the upper part of the photo. Magnification 170 Haematoxylin eosin stain. b) Brain nodule with gemistocyte-like cells. Magnification 4_0 Holzer stain.

Cardiovascular system.

Heart weight 24 g. There were numerous subpericardial petechiae. On the outside of the anterior wall of the left ventricle there were two slight nodules the size of a pea arising from and only slightly paler than the muscle. Three similar pin-head size nodules were present beneath the endocardium on the septal wall of the left ventricle.

Microscopical examination of the nodules disclosed diffusely delimited changes in the muscle fibres, including vacuolisation and fusiform enlargement (Fig. 2a), transverse striations demonstrable t isolated areas only moderate quantities of glycogen, and no fatty deposits.

Respiratory tract. A few petechiae were present on both pleurae. The lungs had the consistency of liver and were dark red in colour. Microscopical areas of atelectasis were demonstrated and, in certain areas, small foci of bronchopneumonia.

Nervous system. The brain was of normal size and no lesions were visible on the sur face of the cortex. Small subependymal swellings p to about 5 mm in diameter were present in both lateral ventricles along the course of the terminal vein (Fig. 2). No other similar changes could be demonstrated at any other site in the brain.

Microscopical examination of the lesions revealed the presence of numerous large cell rich in cytoplasm, some of them multi-nuclear. These were probably of glial type and some resembled gemistocytes (Fig. 2b). The lesions also contained glial cells of usual ppearance and the medulla lso contained isolated, pale large cells, although no such cells were seen in the grey matter of the cortex.

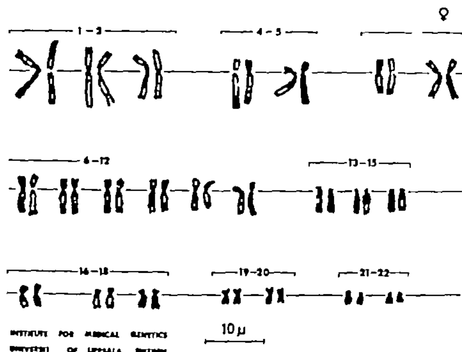


Fig. 4. Karyogram from normal skin of Case 1 showing normal female chromosome complement.

Spleen, lymph nodes and bone marrow Microscopical examination revealed numerous large mono- or polynuclear megakaryocyte-like cells with abundant cytoplasm. Otherwise there were no changes in these organs.

Abdominal viscera and endocrine organs Microscopical examination of the liver showed either isolated, or small groups of large pale cells with abundant cytoplasm. No other significant changes were seen in these organs.

The *placenta* showed a large infarct about 10 cm in diameter and several small infarcts. Histological examination revealed slight vasculitis in the umbilical cord.

Bacteriological investigation of the post mortem material disclosed no definitely pathogenic bacteria.

The morbid-anatomical changes, localized chiefly to the brain and myocardium tally exactly with the changes typical of tuberous sclerosis; the cause of death was due to pulmonary atelectasis and bronchopneumonia. Whether or not these pulmonary lesions may be placed in relation to the primary disease tuberous sclerosis, is impossible to say. Pneumonia would seem to be a far less common cause of death in tuberous sclerosis than the cerebral lesions (including hydrocephalus) or cardiac abnormalities (6).

Chromosome studies in the mother[1]

Biopsies from the apparently normal skin of the left thigh and from a fibroma on the nail bed of a toe from the mother were cultivated according to the standard cell culture technique used at the Institute for Medical Genetics in Uppsala (2). Two and five primary cultures respectively were obtained. Considering the age of the patient (28) the cultures grew normally and good cytological preparations were obtained after 21 days.

[1] This investigation has been aided by a grant from the Foundation Fund for Research in Psychiatry to Professor Jan A. Böök.

The cells derived from the fibroma showed a slightly better growth, probably due to a larger initial explant.

Apparently undamaged cells from both cultures were examined cytologically. The majority of the cells had the normal diploid chromosome number (46) (cf. Table 1)

TABLE 1 *Chromosome counts*

Tissue	Chromosome number 44	45	46 ± 1	46	Total no. of cells
Normal	—	—	3	10	13
Fibroma	1	—	1	30	32

Three very distinct cells derived from the normal skin and five cells derived from the fibroma were matched and analysed in detail. All these cells showed a completely normal chromosome complement (Fig. 4). None of the cultures had an abnormally high frequency of polyploid cells (0–4 per cent).

A 17 year old male with tuberous sclerosis has been karyotyped earlier at the Institute for Medical Genetics in Uppsala. Cells derived from bone marrow showed a normal karyotype with 46 chromosomes and XY complement.

Discussion

The full triad of symptoms was present in the mother and in such cases the diagnosis does not usually present any difficulties. Classical tuberous sclerosis may be overlooked for years however. It is therefore understandable that the first solitary symptoms in a child do not as a rule suggest this disease. In the infant the diagnosis was made solely on the basis of the post mortem findings. To what extent the specific changes of tuberous sclerosis contributed to the child's death is difficult to assess. The probable cause of death was atelectasis and pneumonia presumably secondary to a long and difficult delivery and with some effect of barbiturate in addition. Since it is known that infants dying with this disease during the first years of life usually do so as a result of heart failure and, further since this patient was found to have anomalies of the heart, cardiac failure may well have contributed to the development of the respiratory illness.

The fits in tuberous sclerosis, which commonly first occur before the age of 2 years do not differ in any way from fits of other cause and therefore do not give any clue to the diagnosis except as part of a typical symptom complex. If tuberous sclerosis is to be excluded in a child with fits the physician must base his opinion on other findings. The skin lesions do not acquire the characteristic appearance of sebaceous adenoma until the child has reached school age although other less typical skin anomalies are commonly seen during the first years of life (3). The presence of renal malformations does not as a rule provide a clue as there are often clinically non-specific and silent until so late that the neurological and cutaneous manifestations have already indicated the diagnosis.

Some help can be obtained from roentgenological and electrocardiographic investigations, however. Calcification can occasionally be demonstrated in the cerebral foci, and in not a few cases the presence of foci has been shown by air encephalography (7). Furthermore during recent years typical roentgenological changes in the lungs and bones have been

described in advanced cases (1) A cor responding change phakoma, is seen in the retina. Death from cardiac failure is common (4) in young patients. The myocardial lesions may cause cyanosis enlargement of the heart tachycardia, and electrocardiographic anomalies.

Summary

Changes typical of tuberous sclerosis were observed in a 28-year-old woman and her one-day-old son. The clinical signs in the woman were skin lesions, epileptic fits and low intelligence. In the boy the diagnosis was based on post mortem findings, notably changes in the heart and brain. The diagnostic criteria of this disease are discussed. No chromosomal abnormalities were found either in the normal skin nor in the fibroma on the nail bed of the mother

References

1 Abery B. W Ramage, D Lesions of the vertebrae and innominat bones in tuberous sclerosis. *Brit J Radiol* *30* 274 1957

2 Böök, J A., Fraccaro M., and Lindsten J Cytogenetical observations in mongolism. *Acta Paediat* (*Uppsala*), *48* 453, 1959

3. Chao, D H. C. Congenital neurocutaneous syndromes in childhood. *J Pediat* *45* 447 1959

4. Debré, R., Thieffry S Mozziconacci, P Barbeton E. and Ramade, J La sclérose tubéreuse de Bourneville chez le nourrisson et le petit enfant *Arch Franc Pédiat* *9* 34., 1952.

5 Engelien, D A.: Tuberous sclerosis. *Arch Pediat*, *66* 24, 1949

6. Hallervorden J and Kr cke, W Die tuberöse Hirnsklerose *Handbuch der Spez. Path. Anat. und Histol.* *13/4* 602, 1956.

7 Illing E.: Pathologisch-anatomisch kontrolliert Encephalographien bei tuberöser Sklerose. *Z Ges Neurol Psychiat*, *176* 160, 1943.

8. Landebsteu S.: A case of tuberous sclerosis. *Acta Paediat* (*Uppsala*), *34* 1 1947

9 Marshall, D Saul, G and Sachs, E.: Tuberous sclerosis. *New Engl J Med* *261* 1102, 1959

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PROGRESS IN PEDIATRICS

The Diagnosis and Course of Rheumatoid Arthritis and Benign Aseptic Arthritis in Children[1]

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Arthritis and arthralgias are not rare symptoms among patients in a pediatric ward. In some the etiology is obvious as in cases of *septic* infectious arthritis. In others, however the etiology and pathogenesis may be more obscure the joint affection frequently following an acute upper respiratory infection. In these *aseptic* conditions an allergic-toxic mechanism seems probable and the affection may be labelled postinfectious arthritis. Rheumatic fever can be regarded as an entity within this group the heart involvement being the outstanding feature and the decisive factor for the prognosis. It appears that an allergic-toxic mechanism can also be implicated in arthralgias which occur in some cases of anaphylactoid purpura and in the arthritis, which is one of the extraintestinal manifestations of ulcerative colitis.

Rheumatoid arthritis (RA) can be regarded as an aseptic arthritis of unknown origin which runs a prolonged course The clinical features in children which vary in their pattern from an insidious and undramatic picture to a fullblown, sepsis-like condition, represented by the syndrome of Still and Wissler's subsepsis allergica (28 30 33 34) have been confirmed in several recent reports (8 9 17 22, 25 29) It is generally agreed that most cases in children show certain common trends when compared to adults with the disease. These include the greater tendency for larger joints to be affected early in the course the common acute onset, the relatively high incidence of visceral symptoms and eye lesions, and the difficulties in demonstrating the rheumatoid factor in the serum (15, 36)

The diagnosis in a child presenting a long-standing, symmetrical, aseptic polyarthritis with or without systemic signs hardly offers any difficulties the only problem being to differentiate the condition from other more malignant collagen diseases such as disseminated lupus erythematosus. On the other hand, there may be difficulties at the other end of the spectrum of severity in demarcating RA from other more benign conditions, variously labelled as "postinfectious" arthritis or nonspecific synovitis. These children do not have severe extraarticular systemic

[1] The study was made possible by grant from Forstamsfjblomman Riksförbund mot Ydlingsskolomarna, Gothenburg, Sweden.

manifestations, relapses may occur but the joint process is not destructive and no permanent disability results. It can be claimed that these represent unusually mild cases of RA however it seems more reasonable to demand certain minimal diagnostic criteria in order to be able to compare experiences from different clinics. The well known diagnostic criteria for RA in adults elaborated by the American Rheumatism Association (6 25) are not applicable without modification when dealing with children. In this investigation an attempt has been made to establish criteria special attention being paid to the characteristic features of the disease in childhood and to try to present them in a simplified and perhaps more surveyable way A clinical and prognostic comparison has been made between cases fulfilling these criteria and cases not fulfilling them the latter have in this report been labelled benign aseptic arthritis (BAA)

Prognostic studies of RA in children have yielded varying results, to a large extent depending on a differing selection of the material. Since 1948 the experience from several large clinics has been reported. Bille's series comprised 65 children (4) with a disease of long duration In spit of this the majority improved following chrysotherapy indicating some reversibility of the condition. Schlesinger material (26) consisted of 20 cases with acut onset and high fever showing the features of Still disease Complete recovery with no obvious residual arthritis was the final result in three varying degrees of residual defect remained in 11 four had died and in two the disease was still active. Sury in 195 published a detailed report on 151 cases (29) The mean observation time was 12 years and t the follow-up examination 39 per cent had healed without defects, 32 per cent had residual lesions, not severely handicapping the patient whereas 29 per cent were seriously invalided and 8 per cent had died The prognosis seemed t be worse in cases with an acute onset Barkin also in 195 reported 51 cases (3) with onset before 12 years of age with a remarkable incidence of significant heart disease (31 per cent); Still a severe type of juvenile RA wa present in about 35 per cent. A relatively large proportion of Barkin cases were serious as reflected in his pessimistic dictum once an arthritic always an arthritic" Following an observation time varying from a few up to 40 years after hospitalization, 20 per cent had died, 39 per cent were incapacitated and 41 per cent had a functional recovery permitting performance of all or almost ll normal activity In 1955 Fyf reported on 72 children with RA (12), 56 of whom had a recent follow-up examination. The duration of observation varied from a few to 25 years. In 61 per cent of the examined children the disease had not shown any signs of activity during the preceding two years and approximately two-thirds of these had recovered without sequelae. Less than 10 per cent of those followed-up showed a more significant handicap. An even more optimistic view was expressed by Grokoest and co-workers in 1957 (14) Of their 110 patients examined an average of nine years after onset of the disease, eight had died, 76 per cent of the remainder had recovered, 22 per cent had a slight t moderate handicap and only two per cent were severely incapacitated. Edström & Gedda reported a prognostic study (9) dealing with 90 children. Five of them had the picture of Still syndrome two of whom died, one became invalided and two recovered. The 85 other cases, called juvenile rheumatoid arthritis, showed complete recovery without residual symptoms in 62 per cent whereas 31 per cent showed varying degrees of incapacity One child died and five showed persistent signs of activity rendering a definite evaluation of the outcome difficult. Norcross *et al.* collected 62 cases published in 1958 (21) with an observation time in all of them exceeding seven years. The average duration of activity was 65

[illegible] The Lewis [illegible] follow-up examination [illegible] Gare in five per cent [illegible] Sairanen [illegible] 1958 [illegible] with [illegible] patients with juvenile RA [illegible] represents a selection of more [illegible] and [illegible] cases [illegible] were [illegible] after an average duration of 12 years. [illegible] their monograph [illegible] from different hospitals [illegible] disease was still active in three fourths of the patients. A follow-up examination was performed [illegible] & Pe[illegible] in 1959 reported on their vast experience of more than [illegible] cases of juvenile RA. [illegible] per cent showed a more or less continuous course and [illegible] per cent an intermittent one. Close to half of them had no residual activity of the disease; the active period in these cases had been an average of 7.4 years. One hundred and sixteen of their children had a follow-up examination five years after onset of the disease. The prognosis turned out to be quite favorable with normal functional activity or only a slight handicap in 93 per cent if the patient had been seen by a physician within one year after this onset, 44 per cent were entirely symptom free. If not seen during the first year the corresponding figures were 61 and 13 per cent. From the follow-up 10 years after the onset it was concluded that some further improvement could in general also be seen during the second five-year period.

In the investigations mentioned here the varying results can easily be explained by the differing geographical and social factors, diverging principles of selection [illegible] the [illegible] and [illegible] [illegible].

Present investigation

[illegible] children [illegible] in pediatric departments [illegible] hospitals from [illegible] were [illegible] for a preliminary screening. The [illegible] were eliminated from the [illegible] whose total duration of [illegible] was shorter than three weeks, those where a [illegible] could be proved or seemed probable or where rubella had preceded [illegible] symptoms; children with [illegible] parents [illegible] patients [illegible] other collagen diseases, or when the criteria for the diagnosis of [illegible] fever according to [illegible] were [illegible] remained after these cases had been excluded. During the period [illegible] 1954 to March 1960 eighty-four of these children were examined by the author and an additional three were examined by other physicians. Four children could not be traced or were not willing to cooperate. The group investigated consisted in part of typical cases of rheumatoid arthritis and partly of ones with those of a more benign character. The data from the records were collected to form the basis of a classification which utilized criteria described later in this paper as well as those suggested by the American Rheumatism Association for the diagnosis of rheumatoid arthritis. It must be pointed out however that the charts were incomplete in several instances for the obvious reason that they had not been written with these special criteria in mind. The subsequent course of each patient was analyzed from anamnestic data collected at the time of follow-up, from complete physical examination and, in some cases, from X-ray and laboratory studies.

The main diagnostic grouping of the patients was based on the following criteria

TABLE 4 *Pathogenic microorganisms in nose and throat cultures.*

	Hemolytic streptococci	Pneumococci	Staph. aureus	Haemophilus influenzae	Negative culture
BAA	7	5	10	0	20
RA	6	5	10	1	25

TABLE 5 *Antistreptolysin titer*

Hospitalization	Disease	Less than 200	200–1000	Higher than 1000
Within 3 weeks after onset:	BAA	11	12	1
	RA	12	7	2
Less than 3 weeks after onset:	BAA	8	6	1
	RA	13	5	5
Total	BAA	19	18	2
	RA	25	12	7

TABLE 6 *Antistaphylolysin titer*

Hospitalization	Disease	Less than 2	2–5	Higher than 5
Within 3 weeks after onset	BAA	17	4	3
	RA	11	6	4
Later than 3 weeks after onset:	BAA	11	3	1
	RA	17	5	1
Total	BAA	28	7	4
	RA	28	11	5

TABLE 7 *X ray-examination of sinuses and lungs*

		Sinuses			Lungs		
Hospitalization	Disease	Pathological finding	Normal finding	Not examined	Pathological finding	Normal finding	Not examined
Within 3 weeks after onset	BAA	1	1	19	5	6	11
	RA	2	4	14	3	6	11
Later than 3 weeks after onset	BAA	1	5	12	1	9	8
	RA	2	7	19	1	9	10
Total	BAA	2	6	31	5	15	19
	RA	4	11	33	4	15	20

compared as illustrated in Table 5 and 6 The highest value within one month following the admission has been noted and the groups have again been divided into subgroups, depending on the duration of the symptoms before the admission (irrespective of it was the first onset or a recurrence) X-ray examination of the sinuses and lungs within one week after the admission was undertaken in relatively few children. The results are shown in Table 7 where the same subgrouping has been made. An X ray picture indicating sinusitis was found in approximately one fourth and evidence of a bronchopneumonia in one fifth. No difference between children with BAA or RA was present

In no case had a history indicating a urinary tract infection been given Urinary sediment from specimens obtained soon after admission showed some increase of white cells in five of the patients with RA and in two of those with BAA. When repeated examinations were performed the increase was not constant and urine cultures were sterile.

Trauma.—A history of trauma in the region of the first joint involved within a month preceding the onset was present in one patient with BAA nd in four with RA. The figures are too small to indicate a significant difference.

Monarticular onset —A monarticular onset was relatively common. In the beginning symptoms were localized to a single joint during at least one month in 19 of the 39 BAA cases and in 14 of the 48 RA cases. As time progressed this difference became very marked, only two of the children with RA remained monarticular during the entire period of observation as compared to 17 with BAA.

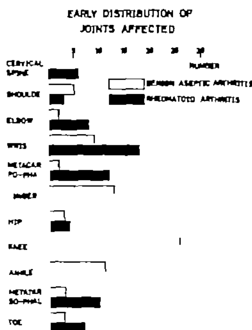


Fig 1. Joints affected in BAA and RA within two months after onset.

Early distribution of affected joints —The distribution of affected joints within two months after the onset is shown in Fig. 1 The knee was most commonly affected in both groups There was a general tendency for more joints to be affected among the RA patients and the symptoms were more often localized to the larger joints in this early stage of the disease. The cervical spine was involved in RA exclusively

Extra-articular manifestations —Visceral manifestations were rare within two months after the onset. Cardiac involvement was present in four of the children with RA a judged by enlargement of the heart on X-ray (one case) and definitely bnormal ECG (three cases) The ECG changes consisted of a lowering of the S–T

TABLE 8 *Duration of fever*

	BAA	RA
Afebrile	22	25
38°C or above maximum seven days	17	21
38 C or above for 8–21 days	0	0
38 C or above for more than 21 days	0	2

interval and abnormally flat T waves. The children with BAA were all normal in this respect None of the RA patients followed a typical course for Still s syndrome and no significant enlargement of the spleen or the lymph nodes was found. There was no evidence of impaired liver function, neither cerebral symptoms nor skin rashes were noted. Involvement of the eyes occurred only among the RA children as iritis (three cases) chorioiditis (two cases) and episcleritis (one case) As mentioned earlier iridocyclitis or uveitis were included among the *minor criteria* for the diagnosis of RA.

Temperature and laboratory findings — A comparison between the two groups as to the degree of fever elevation of the E.S.R. and level of the serum gamma-globulin fraction within two months following the first hospital admission has been made and the findings have been tabulated (Tables 8–10) No significant difference between the two groups was found,

TABLE 9 *Sedimentation rate*

	BAA	RA
0–14 mm	13	6
15–29 mm on at least two occasions	9	17
30 mm or above on at least two occasions	17	25

TABLE 10 *Gamma globulin in serum*

	BAA	RA
Less than 15 %	4	1
15–25 %	3	11
Higher than 25 %	0	1
Not examined	32*	35

but it must be noted that serum electrophoresis was performed in only a minority Had this been done more systematically the tendency to higher gamma globulin values in the RA group might have been demonstrated more definitely A hemoglobin level lower than 10 g per 100 ml during the same early period of the disease was present in eight of the RA and in only one of the BAA patients. During the first two months after the admission the sheep cell agglutination test was performed in 4 of the RA cases, two of them being positive but with low titers (1/64) During the subsequent course additional positive titers appeared in three children (1/64 1/128 1/512)

Course.—The widely different course and the entirely dissimilar prognosis in the two groups has been visualized in various ways. The duration of the disease up to the time of the follow up examination is presented in Table 11 The figures do not as a rule represent the duration of a continuous activity they merely give the span of time during which the symptoms

TABLE 11 *Continuous duration or period (in years) during which relapses occurred*

	0	1	2	3	4	5	6	7–
BAA	21	10	6	1	1	0	0	0
RA	8	8	3	4	4	4	4	11

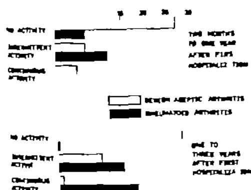


Fig. 3. The course within three years after the first hospitalization.

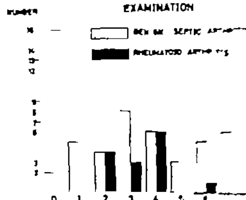


Fig 4. Period preceding the follow-up examination without any activity of the [illegible]

were present either continuously or intermittently The typical pattern for BAA was a single bout of activity of some weeks or a few months duration although recurrences were not rare within the first two years Relapses after this period of time occurred only twice In contrast in more than half of the RA patients, continuous or recurrent activity was present after three or more years. Persistent activity was present or relapses had occurred in 11 cases for as long as 7 years after the onset and it must be remembered that only a part of the material had been observed for this length of time Thus it is probable that the number showing this long-lasting course would increase during prolonged observation.

The course during the first three years fter the first hospital admission has been illustrated in more detail (Fig 3) The more benign progress and the lesser tendency towards continuous activity in the BAA cases is obvious. In Fig 4 the length of time the patient had been free from activity of the disease preceding the follow up is given The varying d[illegible] observation again limits the [illegible] for definite conclusions but [illegible] tic difference between the [illegible] cases is clear

In the patients w[illegible] activity for at least [illegible] follow up examinat[illegible] residual findings w[illegible] The sequelae incl[illegible] movement in the [illegible] of the muscles [illegible] and growth d[illegible] cases showed [illegible]

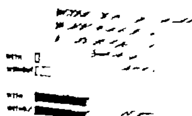


Fig 5 [illegible]

TABLE 12. *Degree of handicap*

	BAA	RA
One year after first hospital admission		
Normal activity	39	38
Partially handicapped	0	8 (17 %)
Severely handicapped	0	2 (4 %)
Three years after first hospital admission		
Normal activity	37	37
Partially handicapped	0	4 (9 %)
Severely handicapped	0	(5 %)

only one of the patients with BAA had residua. The incidence and degree handicap is given in Table 12. Here the functional state of the children has been estimated one and three years, respectively after the first hospital admission. By normal activity is meant that the children were able to take full part in school work and outdoor activities, whereas the partially handicapped had a limited physical capacity which however had not kept them from an essentially normal life. The severely handicapped had either been bedridden, confined to a wheelchair existence or at least had largely been dependent on help for their daily needs. One year after the first hospital admission 21 per cent of the RA patients were handicapped to a varying degree as compared to none of those with BAA. Three years after the first hospital admission the functional state could be evaluated in 37 children with BAA and 43 with RA. The former were again all labelled as having normal activity whereas 14 per cent of the latter were handicapped, one-third of this minority severely handicapped. In one of these the handicap was due entirely to seriously impaired vision, following a bilateral uveitis. It is thus evident that the prognosis is entirely different in BAA as compared to RA also concerning these more severe and largely irreversible conditions. It must, however be pointed out, that the time of observation is too short to establish the definite outcome with respect to the ultimate incidence of a handicap.

Discussion

The criteria presented above for the diagnosis of RA in children need to be commented upon. As previously mentioned the diagnostic criteria elaborated by the American Rheumatism Association do not take into account the special character of the disease in childhood. For reasons of simplicity the present classification has been designed to include a combination of criteria required for making the diagnosis highly probable not, as in A.R.A. classification to establish the diagnosis with different degrees of probability.

The *first* criterion in the present classification deals with signs of arthritis observed by a physician, including all objective findings or symptoms with the requirement of a continuous duration of six weeks. A monarticular RA may occur and is possibly more common in children than in adults. A polyarticular affection is, however more typical especially if it is symmetrical. This is expressed in the *second* criterion. The A.R.A. has the same requirement of duration for the diagnosis of classical or definite RA. In the A.R.A. classification, however it is stated that the interval between the joints becoming affected should not exceed three months. In view of the intermittent course so common in children it has been suggested

that a longer interval should be accepted. Th *third* criterion is a significant agglutination titer indicating the presence of "the rheumatoid factor" It is generally agreed that this is not a common finding in RA in children except possibly when recent methodological modifications are used. This indicates that the low incidence of positive results observed is attributable to the lack of sensitivity of the procedures rather than to an absence of the rheumatoid factor (36) On the other hand, there does not seem to be a higher incidence of nonspecific positive reactions in children, and a positive reaction, although in itself not frequent, must be accepted as valuable support for the diagnosis. Typical X-ray changes constitute the *fourth* criterion and is also found in the A.R.A. classification. What can be regarded as typical may be a matter of dispute but this point has recently been dealt with in detail in a clinico-roentgenological study of RA in children (25). The *fifth* criterion includes all biopsies, which show characteristic changes, including microscopic findings in joint capsules and subcutaneous nodules as described in the notes to the A.R.A. classification (4). These five criteria have been evaluated as being the most important and have consequently been considered as *major criteria*.

The following symptoms and findings have been labelled *minor criteria* If a monarthritis persists for as long as three months or if recurrence has a continuous duration of at least six weeks this is listed as the *first* because it increases the likeliness of a prolonged course and a rheumatoid character of the disease One reason for regarding these prolonged and intermittent cases of monarthritis as truly rheumatoid has been given by Sury (29) who pointed out the similar incidence of eye complications in these patients as compared to those with a more typical polyarticular RA in children. Morning stiffness is a typical finding in RA in adults and is also fairly common in children. The present material can not be used to estimate its incidence however as its importance has not been realized and notes on its occurrence were made only occasionally in the records. It is the author's impression that morning stiffness is not a rare finding in other types of arthritis in children, but there is reason for indicating it as a *second* minor criterion. The *third* is the presence of subcutaneous nodules. They are not frequently encountered and may also occur in acute rheumatic fever The *fourth* is the exanthem variously described by different authors (13 15) as being a common phenomenon in childhood RA. especially among those running a more acute and septic course with high remittent fever. Cases of this type were not seen in this material and no convincing notes describing this rash have been found. Gauchat & May (13), however observed it in 5 to 80 per cent of children with high fever in the initial stages. They described it as maculopapular in character and of a varying distribution similar in appearance to erythema multiforme and called it erythema multiforme rheumatoides. The *fifth* criterion are ocular manifestations more precisely iridocyclitis, uveitis or band-shaped keratitis. It has been pointed out repeatedly (5 8, 9 11 27 32) that these occur more often in children with RA than in adults which justifies their inclusion at least as a minor criterion. In the A.R.A. classification (6, 24) only

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CASE REPORT

Tuberous Sclerosis in a Mother and Her New Born Son

by L. O. BRORSON, K. FREDGA, G. T. HULTQUIST and P. O. LUNDBERG

From the Department of Paediatrics, Institute for Medical Genetics, Department of Pathology and Department of Neurology, The University of Uppsala, Sweden

Tuberous sclerosis or Bourneville's disease is characterized by the symptom triad of sebaceous adenoma, epilepsy and mental deficiency. Studies of families have shown it to be an inherited condition in which morphologically well-defined tumour like foci occur in a number of organs: they can often be strikingly demonstrated in the kidneys, heart and retina. The disease is not confined to any particular germ layer but may affect one or several. The progressive nature of the cerebral lesion is reflected in the increasing degree of mental deficiency and progressive epilepsy: although less characteristic skin lesions have been described, the sebaceous adenomas are regarded as pathognomonic.

The requirement of the above named triad has prevented the diagnosis of tuberous sclerosis in many atypical cases. However, by follow up of these atypical cases it has been possible to trace a simple dominant character in up to 4 generations in many families (3, 8, 9). No hereditary factor has been demonstrated in most of the cases published in the literature, however, and it is probable that isolated cases do occur.

About 10 % of the cases in the literature concern children under 3 years of age and almost all of these have been diagnosed at necropsy (4). This is surprising since tuberous sclerosis is a congenital condition that usually makes its appearance before the age of 9 years (2, 5). It is explained by the facts that the first sign of illness is commonly an epileptic fit; that a mental defect may be difficult to assess in early childhood, that the initial skin lesions are uncharacteristic with regard to appearance and localization, the sebaceous adenomas not becoming apparent until a later date; and that the full clinical picture develops gradually and progresses slowly with increasing age.

The present paper is devoted to an account of 2 cases of tuberous sclerosis, in a mother and her child. They are interesting because of the direct inheritance which they illustrate, and because post-mortem examination of the infant who was only one day old, revealed lesions typical of tuberous sclerosis.

Case 1. M.M.B. (Record no 144/60, Dept of Neurology). The patient was a woman of 26 years. Her parents were unmarried and the family history is therefore uncer-



Fig. 1 Case 1) Sebaceous adenoma in the face b) Paraungual fibroma and nail changes.) Gingival h perplasia

Fig. — Case 2. Subependymal nodule in the wall of one of the lateral ventricles. Magnification 10

n. Her father cousin was said to have major epileptic fits. See below regarding the patient son.

The patient had an 8-year elementary chool education, after which she had orked as housemaid until she married at 7 years of age.

Past illnesses included infectious hepatitis the age of 10 years. Since the menarche ad not occurred t 17 years, hormone treat nent was instituted and continued for 2 ears at which time the periods became egular and pparently normal.

From about the age of 17 the patient suf from fits, thus, these made their p- earance at bout the time hormone treat ent as commenced. The patient states e fits start with dizziness and feeling of f inting; she has no memory of what happens during the next 30–60 seconds. Although the ttacks may occur at any time, for example when the patient is drying dishes, when she is out cycling, or when attending t a child, she is ble to continue her work, the only outward sign being pallor and flushing of the face. Without treatment she had up to about 10 fits per day but following treatment with phenantoin and phenobarbital which was started in 1953 they have occurred only at long intervals. There have never been convulsions during these fits; she has never fallen to the ground, has never been incontinent of urine or faeces, nor has she ever bitten her tongue.

The patient became pregnant in 1959 the date of her last menstrual period being

March 31 After an essentially normal preg nancy she was delivered on January 22, 1960, about 2 weeks after the expected date of confinement During and after delivery the patient lost 1800 g of blood. The pla centa, which was rigid, was manually removed

On admission t the neurological depart ment on April 19 1960 the patient was obese and of average height. Over the bridge of the nose, the alae nasae, and the cheeks adjacent to the nose were a number of yellowish white swellings up to the size of a rice grain (Fig 1a), the ppearance of which was typical of sebaceous adenoma. There were a number paraungual reddish soft swellings up to the size of a pea (Fig. 1b) At the level of each tumour there was a deep groove in the nail itself The morphology of the swellings proved to be the same as that seen in paraungual fibromata. The papillae of the tongue were slightly enlarged. The gum behind the upper incisors was deeply and irregularly lobulated, the sur face having the appearance of normal gum mucosa (Fig. 1c) The histological appearance was that of non-specific hyperplasia and inflammation, and was probably not due to tuberous sclerosis (the patient had received phenantoin) The hard palate was unusually high and narrow but otherwise there were no abnormal findings in the ears, nose, or throat The distribution of hair was normal. The abdomen was rather adipose, with striae gravidarum. No abnormalities of the internal organs were noted. The results of oto-neurological and ophthalmo-neurological examination were normal.

Experimental psychological examination showed that the patient s capacity in most of the tests was under the average The pa tient s intelligence was obviously subnormal.

The EEG was pathological, with moderately marked, continuous, non-specific abnormality in the right fronto-temporal region.

X-ray of the skull, including tomography disclosed slight to moderate hyperostosis frontalis interna. Within the right frontal region there were extensive plaque-like patches of calcification in an area slightly larger than a walnut There were also suspi cions of similar scattered plaques in both parieto-temporal regions.

X-rays of the chest and abdomen were normal. The ECG was normal. The routine blood and urine tests gave normal results except for slight anaemia (Hb 10 7 g%). The Wassermann, Kline and Meinicke reactions were negative. The Sabin Feldman dye test and the complementfixation test for to oplasmosis were negative The patent's bloodgroup was O D-negative.

Case 2 (Record no 120/60, Dept. of Paediatrics) This patient a boy was the first and only child of Case 1 The expected date of birth was January 7 1960 he was born on January 22, 1960, in right occipito-anterior presentation, and weighed 3,840 g. The amniotic fluid was deeply stained with meconium. The mother had fits during labour and was therefore given pethidine (50 mg) and sodium amobarbital (0.2 mg). Slowing of the foetal heart was noted several times during the hours before birth.

Immediately after birth the infant was in good condition after aspiration of mucous. An attack of deep cyanosis took place 1½ hours after birth, and he was transferred t the paediatric clinic. On admission to the ward he was cyanotic and hypotonic. Respirations were shallow and infrequent, with dyspnoea and a piercing cry Temperature 32.5°C. There were no skin lesions. Large quantities of mucous were aspirated. The breath sounds were abnormal with crepitations on both sides. Repeated ttacks of cyanosis took place during the next few hours, and the infant died during such an attack 11 hours after birth.

Laboratory tests. Estimation of barbiturate as amobarbital in certain organs post mortem: serum 0.8 mg/100 ml; brain 0.0043 mg/g wet weight; liver 0 023 mg/g wet weight.

Necropsy (Record no 56/60 Dept. of Pathology)



Fig. 2. Microphot of the myocardial and cerebral changes in Case 2.) Myocardium with changed muscle fibers in the upper part of the photo. Magnification 170 Haematoxylin eosin stain. b) Brain nodule with gemistocyte-like cells. Magnification 450 Holzer stain.

Cardiovascular system

Heart weight 24 g There were numerous subpericardial petechiae On the outside of the anterior wall of the left ventricle there were two slight nodules the size of a pea arising from and only slightly paler than the muscle Three similar pin-head size nodules were present beneath the endocardium on the septal wall of the left ventricle

Microscopical examination of the nodules disclosed diffusely delimited changes in the muscle fibres, including vacuolization and fusiform enlargement (Fig 3), transverse striations demonstrable at isolated areas only moderate quantities of glycogen, and no fatty deposits.

Respiratory tract. A few petechiae were present on both pleurae The lungs had the consistency of liver and were dark red in colour Microscopical areas of telectasis were demonstrated and, in certain areas, small foci of bronchopneumonia.

Nervous system. The brain was of normal size, and no lesions were visible on the sur face of the cortex Small subependymal swellings up to about 5 mm in diameter were present in both lateral ventricles along the course of the terminal vein (Fig 2) No other similar changes could be demonstrated at any other site in the brain.

Microscopical examination of the lesions revealed the presence of numerous large cells rich in cytoplasm, some of them multi nuclear These were probably of glial type, and some resembled gemistocytes (Fig 3b). The lesions also contained glial cells of usual ppearance; and the medulla also contained isolated, pale large cells, although no such cells were seen in the grey matter of the cortex.

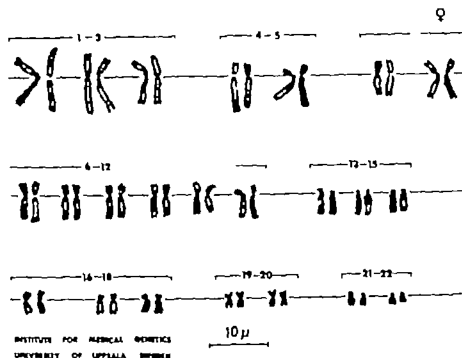


Fig 4 Karyogram from normal skin of Case 1 showing normal female chromosome complement.

Spleen lymph nodes and bone-marrow Microscopical examination revealed numerous large mono- or polynuclear megakaryocyte-like cells with bundant cytoplasm. Otherwise there were no changes in these organs.

Abdominal viscera and endocrine organs Microscopical examination of the liver showed either isolated or small groups of large, pale cells with bundant cytoplasm. No other significant changes were seen in these organs.

The *placenta* showed large infarct bout 10 cm in diameter and several small infarcts. Histological examinat on revealed slight vasculitis in the umbilical cord.

Bacteriological *vestigation* of the post mortem material disclosed no definitely pathogenic bacteria.

The morbid-anatomical changes localized chiefly to the brain and myocardium, tally exactly with the changes typical of tuberous sclerosis the cause of death was due to pulmonary telectasis and bronchopneumonia. Whether or not these pulmonary lesions may be placed in relation t the primary disease, tuberous sclerosis, is impossible to say. Pneumonia would seem to be a far less common cause of death in tuberous sclerosis than the cerebral lesions (including hydrocephalus) or cardiac bnormalities (6).

Chromosome studies in the mother[1]

Biopsies from the pparently normal skin of the left thigh and from a fibroma on the nail-bed of a toe from the mother were cultivated according to the standard cell culture technique used at the Institute for Medical Genetics in Uppsala (2). Two and five primary cultures respectively were obtained. Considering the age of the patient (28) the cultures grew normally and good cytological preparations were obtained after 21 days.

[1] This investigation has been aided by grant from the Foundation's Fund for Research in Psychiatry t Professor Jan A. Böök.

The cells derived from the fibroma showed a slightly better growth, probably due to a larger initial explant.

Apparently undamaged cells from both cultures were examined cytologically. The majority of the cells had the normal diploid chromosome number (46) (cf. Table 1).

TABLE 1. *Chromosome counts*

Tissue	Chromosome number 44	45	46±1	46	Total no. of cells
Normal	—	—	3	10	13
Fibroma	1	—	1	30	32

Three very distinct cells derived from the normal skin and five cells derived from the fibroma were matched and analysed in detail. All these cells showed a completely normal chromosome complement (Fig. 4). None of the cultures had an abnormally high frequency of polyploid cells (0–4 per cent).

A 17 year old male with tuberous sclerosis has been karyotyped earlier at the Institut for Medical Genetics in Uppsala. Cells derived from bone marrow showed a normal karyotype with 46 chromosomes and XY complement.

Discussion

The full triad of symptoms was present in the mother and in such cases the diagnosis does not usually present any difficulties. Classical tuberous sclerosis may be overlooked for years, however. It is therefore understandable that the first solitary symptoms in a child do not as a rule suggest this disease. In the infant the diagnosis was made solely on the basis of the post mortem findings. To what extent the specific changes of tuberous sclerosis contributed to the child's death is difficult to assess. The probable cause of death was atelectasis and pneumonia, presumably secondary to a long and difficult delivery and with some effect of barbiturate in addition. Since it is known that infants dying with this disease during the first years of life usually do so as a result of heart failure and, further, since this patient was found to have anomalies of the heart, cardiac failure may well have contributed to the development of the respiratory illness.

The fits in tuberous sclerosis, which commonly first occur before the age of 2 years, do not differ in any way from fits of other cause and therefore do not give any clue to the diagnosis except as part of a typical symptom complex. If tuberous sclerosis is to be excluded in a child with fits the physician must base his opinion on other findings. The skin lesions do not *acquire the characteristic appearance of* sebaceous adenoma until the child has reached school age, although other less typical skin anomalies are commonly seen during the first years of life (3). The presence of renal malformations does not as a rule provide a clue, as these are often clinically non-specific and silent until so late that the neurological and cutaneous manifestations have already indicated the diagnosis.

Some help can be obtained from roentgenological and electrocardiographic investigations, however. Calcification can occasionally be demonstrated in the cerebral foci, and in not a few cases the presence of foci has been shown by air encephalography (7). Furthermore, during recent years typical roentgenological changes in the lungs and bones have been

described in advanced cases (1) A corresponding change, phakoma, is seen in the retina. Death from cardiac failure is common (4) in young patients. The myocardial lesions may cause cyanosis, enlargement of the heart, tachycardia, and electrocardiographic anomalies.

Summary

Changes typical of tuberous sclerosis were observed in a 28-year-old woman and her one-day-old son. The clinical signs in the woman were skin lesions, epileptic fits and low intelligence. In the boy the diagnosis was based on post mortem findings, notably changes in the heart and brain. The diagnostic criteria of this disease are discussed. No chromosomal abnormalities were found either in the normal skin nor in the fibroma on the nail bed of the mother

References

1 Ashby B. W Ramage, D Lesions of the vertebrae and innominate bones in tuberous sclerosis. *Brit J Radiol*, *30* 274, 1957
2. Böök, J A Fraccaro, M., and Lindsten J Cytogenetical observations in mongolism. *Acta Pædiat* (*Uppsala*), *48* 453 1959
3 Chao D H. C.: Congenital neurocutaneous syndromes in childhood. *J Pediat*, *55* 447 1959
4. Debré, R., Thieffry S., Mozziconacci, P Bargeton E. and Ramade, J La sclérose tubéreuse de Bourneville chez le nourrisson et le petit enfant *Arch Franc Pédiat*, 9 342 1952.
5. Englisch, D A.: Tuberous sclerosis. *Arch Pediat*, *66* 24 1949.
6. Hallervorden J and Krücke, W *Die* tuberöse Hirnsklerose *Handbuch der Spez Path. Anat und Histol*, *13/4* 602, 1956.
7 Illing, E.: Pathologisch-anatomisch kontrollierte Encephalographien bei tuberöser Sklerose. *Z Ges Neurol Psychiat* *176* 160, 1943.
8. Lardinsuu S.: A case of tuberous sclerosis. *Acta Pædiat* (*Uppsala*), *34* 1 1947
9. Marshall, D Saul, G. and Sachs, E Tuberous sclerosis. *New Engl J Med* *261* 1102, 1959.

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PROGRESS IN PEDIATRICS

The Diagnosis and Course of Rheumatoid Arthritis and Benign Aseptic Arthritis in Children[1]

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Arthritis and arthralgias are not rare symptoms among patients in a pediatric ward. In some the etiology is obvious as in cases of *septic* infectious arthritis. In others, however, the etiology and pathogenesis may be more obscure, the joint affection frequently following an acute upper respiratory infection. In these *aseptic* conditions an allergic-toxic mechanism seems probable and the affection may be labelled postinfectious arthritis. Rheumatic fever can be regarded as an entity within this group, the heart involvement being the outstanding feature and the decisive factor for the prognosis. It appears that an allergic toxic mechanism can also be implicated in arthralgias which occur in some cases of anaphylactoid purpura and in the arthritis, which is one of the extraintestinal manifestations of ulcerative colitis.

Rheumatoid arthritis (RA) can be regarded as an aseptic arthritis of unknown origin which runs a prolonged course. The clinical features in children which vary in their pattern from an insidious and undramatic picture to a fullblown, sepsis-like condition, represented by the syndrome of Still and Wissler's "subsepsis allergica" (28, 30, 33, 34) have been confirmed in several recent reports (8, 9, 17, 22, 25, 29). It is generally agreed that most cases in children show certain common trends when compared to adults with the disease. These include the greater tendency for larger joints to be affected early in the course, the common acute onset, the relatively high incidence of visceral symptoms and eye lesions, and the difficulties in demonstrating the rheumatoid factor in the serum (35, 36).

The diagnosis in a child presenting a long-standing, symmetrical aseptic polyarthritis with or without systemic signs hardly offers any difficulties, the only problem being to differentiate the condition from other more malignant collagen diseases such as disseminated lupus erythematosus. On the other hand, there may be difficulties at the other end of the spectrum of severity in demarcating RA from other more benign conditions, variously labelled as "postinfectious" arthritis or nonspecific synovitis. These children do not have severe extraarticular systemic

[1] The study was made possible by a grant from Förstamajblommans Riksförbund mot Folksjukdomarna, Gothenburg, Sweden.

manifestations relapses may occur but the joint process is not destructive and no permanent disability results. It can be claimed that these represent unusually mild cases of RA, however it seems more reasonable to demand certain minimal diagnostic criteria in order to be able to compare experiences from different clinics. The well known diagnostic criteria for RA in adults elaborated by the American Rheumatism Association (6 25) are not applicable without modification when dealing with children In this investigation an attempt has been made to establish criteria special attention being paid to the characteristic features of the disease in childhood and to try to present them in a simplified and perhaps more surveyable way A clinical and prognostic comparison has been made between cases fulfilling these criteria and cases not fulfilling them the latter have in this report been labelled benign aseptic arthritis (BAA)

Prognostic studies of RA in children have yielded varying results, to a large extent depending on a differing selection of the material Since 1946 the experience from several large clinics has been reported Bille series comprised 65 children (4) with a disease of long duration In spite of this the majority improved following chrysotherapy indicating some reversibility of the condition Schlesinger material (26) consisted of 20 cases with acute onset and high fever showing the features of Still disease Complete recovery with no obvious residual arthritis was the final result in three varying degrees of residual defect remained in 11 four had died and in tw the disease was still active. Bury in 195.. published detailed report on 151 cases (29) The mean observ tion time was 12 years and at the follow up examination 39 per cent had healed without defects, 22 per cent had residual lesions, not severely handicapping the patient, whereas 29 per cent were seriously invalided and 8 per cent had died. The prognosis seemed to be worse in cases with an acute onset Barkin also in 1952 reported 51 cases (3) with onset before 12 years of age with a remarkable incidence of significant heart disease (31 per cent); Still's severe type of juvenile RA was present in about 35 per cent. A relatively large proportion of Barkin's cases were serious as reflected in his pessimistic dict un once an arthritic always an arthritic Following an observation time varying from a few up t 40 years after hospitalization, 20 per cent had died 39 per cent were incapacitated and 41 per cent had a functional recovery permitting performance of all or almost all normal activity In 1955 Pyf reported on 72 children with RA (12), 56 of whom had a recent follow-up examination. The duration of observation varied from a few to ..5 years. In 61 per cent of the examined children the disease had not shown any signs of activity during the preceding two years and approximat ly two-thirds of these had recovered without sequelae. Less than 10 per cent of those followed-up showed a more significant handicap. An even more optimistic view was expressed by Grokoest and co-workers in 1957 (14). Of their 110 patient examined an average of nine years after onset of the disease, eight had died, 76 per cent of the remainder had recovered, 22 per cent had a slight to moderat handicap and only two per cent were severely incapacitated. Edström & Gedda reported a prognostic study (9) dealing with 90 children. F ve of them had the picture of Still s syndrome two of whom died, one became invalided and two recovered The 85 other cases, called juvenile rheumatoid arthritis, showed complete recovery without residual symptoms in 6.. per cent whereas 31 per cent showed varying degrees of incapacity One child died and five showed persistent signs of activity rendering a definite evaluation of the outcome difficult. Norcross et al. collected 62 cases published in 1958 (21) with an observation time in all of them exceeding seven years. The average duration of activity was 44

months Two-thirds had only one period of activity but recurrences had been observed following an interval of 10 years. The disease was inactive in 73 per cent at the follow-up examination. Two thirds had recovered entirely or almost completely the rest showing varying degrees of handicap Close to five per cent had died. The 16 children with the clinical picture of Still's syndrome had the worst prognosis. Sauranen, in 1958, published a report dealing mainly with the roentgenological aspects of 100 patients with juvenile RA (25) His material apparently represents a selection of more chronic and disabled cases. Twenty two were considered arrested and 15 of these were largely incapacitated after an average duration of 13 years. Köttgen & Callensee in their monograph (18), based on inquiries from different hospitals, found that at discharge the disease was still active in three fourths of the patients. No follow-up examination was performed. Ansell & Bywaters, in 1959 reported on their vast experience of more than 200 cases of juvenile RA (2) Thirty-one per cent showed a more or less continuous course and 22 per cent an intermittent one Close to half of them had no residual activity of the disease; the active period in these cases had been an average of 7 4 years. One hundred and sixteen of their children had a follow-up examination five years after onset of the disease The prognosis turned out to be quite favorable with normal functional activity or only a slight handicap in 98 per cent if the patients had been seen by a physician, within one year after this onset 44 per cent were entirely symptom free If not seen during the first year the corresponding figures were 61 and 13 per cent. From the follow up 10 years after the onset it was concluded that some further improvement could in general also be seen during the second five-year period

In the investigations mentioned above the varying results can easily be explained by the differing geographical and social factors diverging principles of selection and heterogenous classification. The distinction from other types of aseptic arthritis has sometimes not been well defined.

Present investigation

Records of children with arthritis treated in pediatric departments of four Stockholm hospitals from 195 to 1957 were collected for a preliminary screening The following were eliminated from the study patients whose total duration of joint symptoms was shorter than three weeks; those where a septic etiology could be proved or seemed probable or where rubella had preceded the symptoms; children with allergic purpura, ulcerative colitis, psoriasis, leukemia, other collagen diseases, or when the criteria for the diagnosis of rheumatic fever according to Wallgren 1957 (31) were fulfilled. Ninety one remained after these cases had been excluded. During the period July 1959 to March 1960 eighty four of these children were examined by the author; an additional three were examined by other physicians. Four children could not be traced or were not willing to cooperate. The group investigated consisted in part of typical cases of rheumatoid arthritis and partly of aseptic arthritis of more benign character The data from the records were collected to formulate a classification which utilized criteria described later in this paper as well as those suggested by the American Rheumatism Association for the diagnosis of rheumatoid arthritis. It must be pointed out, however that the charts were incomplete in several instances for the obvious reason that they had not been written with these special criteria in mind. The subsequent course of each patient was analyzed from anamnestic data collected at the time of follow up, from a complete physical examination and, in some cases, from X-ray and laboratory studies.

The main diagnostic grouping of the patients was based on the following criteria.

Major criteria

1 Objective arthritis symptoms from one joint observed by a physician and of a continuous duration of at least six weeks

2 One or several additional joints affected according to the same qualification and with the same duration. An interval exceeding three months between the time the first and subsequent joints were affected was accepted.

3 Significant agglutination titer indicating presence of the rheumatoid factor"

4 Typical X ray changes.

5 Biopsy showing characteristic changes.

Minor criteria

1 Continuing active arthritis of one joint observed by a physician for at least three months totally or a recurrence with a duration of at least six weeks.

2 Morning stiffness.

3. Subcutaneous nodules.

4 Erythema multiforme rheumatoides.

5. Iridocyclitis, uveitis or band-shaped keratitis.

Exclusions

Findings indicating a septic, tuberculous or viral etiology salmonella infection, urinary tract infection, disseminated lupus erythematosus periarteritis nodosa, leukemia ulcerative colitis, anaphylactoid purpura, psoriasis, agammaglobulinemia, rheumatic fever

Two major criteria or one major and two minor were required for the diagnosis of rheumatoid arthritis presupposing that exclusion factors were absent. Cases in this investigation not fulfilling these criteria were called benign aseptic arthritis.

Results

Of the 87 patients, seen in a follow up examination, 48 fulfilled the diagnostic criteria of rheumatoid arthritis (RA) the number labelled benign aseptic arthritis (BAA) was 39 These two groups were compared as to the clinical picture and course

Heredity—The incidence of different types of rheumatic diseases among relatives is shown in Table 1 The incidence was similar in the two groups.

Sex ratio.—The preponderance for girls is shown in Table 2 and was of similar magnitude in both groups

Age—The age at onset is illustrated in Fig 1 It is clear from this that in the RA group the disease had an earlier onset,

TABLE 1 *Heredity for rheumatic diseases*

	Mother	Father	Siblings	Other relatives (grand parents, uncles, aunts, cousins)
BAA (39 cases)				
Acute polyarthritis rheumatic fever benign aseptic arthritis	2	2	0	6
Rheumatoid arthritis	0	0	0	3
Non-classifiable	1	0	0	0
Positive heredity for all types in 21				
RA (48 cases).				
Acute polyarthritis rheumatic fever benign aseptic arthritis	3	0	0	2
Rheumatoid arthritis	1	0	0	4
Non-classifiable	0	0	0	2
Positive heredity for all types in [illegible]				

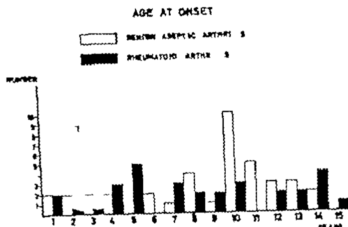


Fig. 1. Age at onset in BAA and RA.

TABLE 2. *Sex distribution.*

	♀	♂	♀/♂
BAA	24	12	2/1
RA	31	17	1.8/1

being most common before 5 with a peak between and 3 years of age. In contrast BAA occurred more commonly in older children, the peak appearing between 10 and 11 years of age.

Preceding infection.—A history of an upper respiratory infection preceding the joint symptoms was a common finding in both groups. Such a history within four weeks before the onset occurred in 15 of the 39 BAA group and in 14 of the 48 children with RA. In order to analyze the importance of a preceding infection in more detail a further comparison was made regarding evidence of the association. Among children admitted within 3 weeks of the onset or recurrence of joint symptoms only a few in both groups had significant findings of an acute upper respiratory infection. Nose and throat cultures were performed in most of the children and the findings have been summarized in Table 3 and 4. There did not seem to be any difference as to the incidence of pathogenic organisms in the two groups, nor was the incidence influenced by the variable length of the time having elapsed between the onset of the symptom and the admission to the hospital. It is also seen that the incidence of various pathogenic organisms was almost identical in the two groups. The antistreptolysin and antistaphylolysin titers have also been

TABLE 3. *Nose and throat cultures.*

Hospitalization	Disease	Pathological finding	Normal finding
Within 3 weeks after onset	BAA	11	11
	RA	9	12
Later than 3 weeks after onset	BAA		8
	RA	11	11
Total	BAA	18	19
	RA	20	23

TABLE 4 *Pathogenic microorganisms in nose and throat cultures*

	Hemolytic streptococci	Pneumococci	Staph. aureus	Haemophilus influenzae	Negative culture
BAA	7	5	10	0	20
RA	6	5	10	1	25

TABLE 5 *Antistreptolysin titer*

Hospitalization	Disease	Less than 200	200-1000	Higher than 1000
Within 3 weeks after onset:	BAA	11	12	1
	RA	12	7	2
Less than 3 weeks after onset:	BAA	8	6	1
	RA	13	5	5
Total	BAA	19	18	
	RA	25	12	7

TABLE 6 *Antistaphylolysin titer*

Hospitalization	Disease	Less than 2	–5	Higher than 5
Within 3 weeks after onset	BAA	17	4	3
	RA	11	6	4
Later than 3 weeks after onset	BAA	11	3	1
	RA	17	5	1
Total	BAA	28	7	4
	RA	28	11	5

TABLE 7 *X ray-examination of sinuses and lungs*

		Sinuses			Lungs		
Hospitalization	Disease	Pathological finding	Normal finding	Not examined	Pathological finding	Normal finding	Not examined
Within 3 weeks after onset	BAA	1	1	10	8	6	11
	RA	2	4	14	3	6	11
Later than 3 weeks after onset	BAA	1	5	12	1	9	8
	RA	2	7	19	1	9	18
Total	BAA		6	31	6	15	19
	RA	4	11	33	4	15	29

compared as illustrated in Table 5 and 6 The highest value within one month following the admission has been noted and the groups have again been divided into subgroups, depending on the duration of the symptoms before the admission (irrespective of it was the first onset or a recurrence) X ray examination of the sinuses and lungs within one week after the admission was undertaken in relatively few children. The results are shown in Table 7 where the same subgrouping has been made An X ray picture indicating sinusitis was found in approximately one fourth and evidence of a bronchopneumonia in one fifth. No difference between children with BAA or RA was present.

In no case had a history indicating a urinary tract infection been given Urinary sediment from specimens obtained soon after admission showed some increase of white cells in five of the patients with RA and in two of those with BAA When repeated examinations were performed the increase was not constant and urine cultures were sterile.

Trauma —A history of trauma in the region of the first joint involved within a month preceding the onset was present in one patient with BAA and in four with RA. The figures are too small to indicate a significant difference.

Monarticular onset —A monarticular onset was relatively common. In the beginning symptoms were localized to a single joint during at least one month in 19 of the 30 BAA cases and in 14 of the 48 RA cases. As time progressed this difference became very marked, only two of the children with RA remained monarticular during the entire period of observation as compared to 17 with BAA.

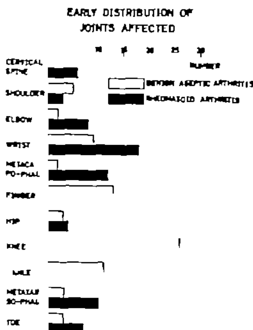


Fig. 2. Joints affected in BAA nd RA within two months after onset.

Early distribution of affected joints — The distribution of affected joints within two months after the onset is shown in Fig 1 The knee was most commonly affected in both groups. There was a general tendency for more joints to be affected among the RA patients and the symptoms were more often localized to the larger joints in this early stage of the disease The cervical spine was involved in RA exclusively

Extra-articular manifestations —Visceral manifestations were rare within two months after the onset. Cardiac involvement was present in four of the children with RA, as judged by enlargement of the heart on X ray (one case) and definitely abnormal ECG (three cases) The ECG changes consisted of a lowering of the S–T

Table 8 *Duration of fever*

	BAA	RA
Afebrile	22	25
38 C or above maximum seven days	17	21
38 C or above for 8–21 days	0	0
38 C or above for more than 21 days	0	2

interval and abnormally flat T waves. The children with BAA were all normal in this respect None of the RA patients followed a typical course for Still's syndrome and no significant enlargement of the spleen or the lymph nodes was found There was no evidence of impaired liver function, neither cerebral symptoms nor skin rashes were noted. Involvement of the eyes occurred only among the RA children as iritis (three cases), chorioiditis (two cases) and episcleritis (one case) As mentioned earlier iridocyclitis or uveitis were included among the minor criteria for the diagnosis of RA

Temperature and laboratory findings — A comparison between the two groups as to the degree of fever elevation of the E.S.R and level of the serum gamma globulin fraction within two months following the first hospital admission has been made and the findings have been tabulated (Tables 8–10) No significant difference between the two groups was found,

Table 9 *Sedimentation rate*

	BAA	RA
0–14 mm	13	6
15–29 mm on at least two occasions	9	17
30 mm or above on at least two occasions	17	25

Table 10 *Gamma globulin in serum*

	BAA	RA
Less than 15 %	4	1
15–25 %	3	11
Higher than 25 %	0	1
Not examined	22	23

but it must be noted that serum electrophoresis was performed in only a minority Had this been done more systematically the tendency to higher gamma globulin values in the RA group might have been demonstrated more definitely A hemoglobin level lower than 10 g per 100 ml during the same early period of the disease was present in eight of the RA and in only one of the BAA patients. During the first two months after the admission the sheep cell agglutination test was performed in [illegible] of the RA cases, two of them being positive but with low titers (1/64) During the subsequent course additional positive titers appeared in three children (1/64, 1/128 1/51)

Course —The widely different course and the entirely dissimilar prognosis in the two groups has been visualized in various ways. The duration of the disease up to the time of the follow up examination is presented in Table 11 The figures do not as a rule represent the duration of a continuous activity they merely give the span of time during which the symptoms

Table 11 *Continuous duration or period (in years) during which relapses occurred*

	0	1	2	3	4	5	6	7
BAA	21	10	6	1	1	0	0	0
RA	8	8	5	4	4	4	4	11

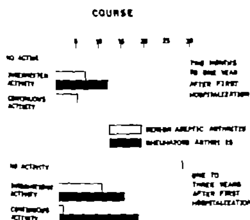


Fig. 3. The course within three years after the first hospitalization.

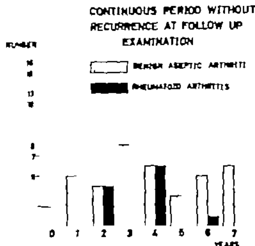


Fig. 4. Period preceding the follow-up examination without any activity of the joint symptoms.

were present either continuously or intermittently. The typical pattern for BAA was a single bout of activity of some weeks or a few months duration although recurrences were not rare within the first two years. Relapses after this period of time occurred only twice. In contrast in more than half of the RA patients, continuous or recurrent activity was present after three or more years. Persistent activity was present or relapses had occurred in 11 cases for as long as 7 years after the onset and it must be remembered that only a part of the material had been observed for this length of time. Thus it is probable that the number showing this long-lasting course would increase during prolonged observation.

The course during the first three years after the first hospital admission has been illustrated in more detail (Fig. 3). The more benign progress and the lesser tendency towards continuous activity in the BAA cases is obvious. In Fig. 4 the length of time the patients had been free from activity of the disease preceding the follow up is given. The varying duration of observation again limits the possibilities for definite conclusions, but the systematic difference between the two groups of cases is clear.

In the patients who had no signs of activity for at least one year preceding the follow-up examination the incidence of residual findings was compared (Fig. 5). The sequelae include defective range of movement in the affected joints, atrophy of the muscles, deformities, contractures and growth disturbance. Half of the RA cases showed residual defects, whereas

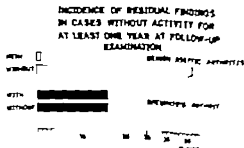


Fig. 5. Incidence of residual findings in BAA and RA at the follow-up examination.

TABLE 1° *Degree of handicap*

	BAA	RA
One year after first hospital admission		
Normal activity	39	38
Partially handicapped	0	8 (17 %)
Severely handicapped	0	2 (4 %)
Three years after first hospital admission		
Normal activity	37	37
Partially handicapped	0	4 (9 %)
Severely handicapped	0	2 (5 %)

only one of the patients with BAA had residua. The incidence and degree handicap is given in Table 1° Here the functional state of the children has been estimated one and three years, respectively after the first hospital admission By normal activity is meant that the children were able to take full part in school work and outdoor activities, whereas the partially handicapped had a limited physical capacity which however had not kept them from an essentially normal life. The severely handicapped had either been bedridden, confined to a wheelchair existence or at least had largely been dependent on help for their daily needs. One year after the first hospital admission 21 per cent of the RA patients were handicapped to a varying degree as compared to none of those with BAA. Three years after the first hospital admission the functional state could be evaluated in 37 children with BAA and 43 with RA. The former were again all labelled as having normal activity whereas 14 per cent of the latter were handicapped, one third of this minority severely handicapped. In one of these the handicap was due entirely to seriously impaired vision, following a bilateral uveitis. It is thus evident that the prognosis is entirely different in BAA as compared to RA also concerning these more severe and largely irreversible conditions. It must however be pointed out, that the time of observation is too short to establish the definite outcome with respect to the ultimate incidence of a handicap

Discussion

The criteria presented above for the diagnosis of RA in children need to be commented upon. As previously mentioned the diagnostic criteria elaborated by the American Rheumatism Association do not take into account the special character of the disease in childhood. For reasons of simplicity the present classification has been designed to include a combination of criteria required for making the diagnosis highly probable· not as in A.R.A. classification to establish the diagnosis with different degrees of probability

The *first* criterion in the present classification deals with signs of arthritis observed by a physician, including all objective findings or symptoms with the requirement of a continuous duration of six weeks. A monarticular RA may occur and is possibly more common in children than in adults. A polyarticular affection is however more typical especially if it is symmetrical This is expressed in the *second* criterion. The A.R.A. has the same requirement of duration for the diagnosis of classical or definite RA In the A.R.A. classification, however it is stated that the interval between the joints becoming affected should not exceed three months. In view of the intermittent course so common in children it has been suggested

that a longer interval should be accepted. The *third* criterion is a significant agglutination titer indicating the presence of "the rheumatoid factor" It is generally agreed that this is not a common finding in RA in children except possibly when recent methodological modifications are used. This indicates that the low incidence of positive results observed is attributable to the lack of sensitivity of the procedures rather than to an absence of the rheumatoid factor (36) On the other hand, there does not seem to be a higher incidence of nonspecific positive reactions in children, and a positive reaction, although in itself not frequent, must be accepted as valuable support for the diagnosis. Typical X ray changes constitute the *fourth* criterion and is also found in the A.R.A. classification. What can be regarded as typical may be a matter of dispute, but this point has recently been dealt with in detail in a linico-roentgenological study of RA in children (25) The *fifth* criterion includes all biopsies which show characteristic changes including microscopic findings in joint capsules and subcutaneous nodules as described in the notes to the A.R.A. classification (24) These five criteria have been evaluated as being the most important and have consequently been considered as *major criteria*

The following symptoms and findings have been labelled *minor criteria*. If a monarthritis persists for as long as three months or if a recurrence has a continuous duration of at least six weeks, this is listed as the *first* because it increases the likeliness of a prolonged course and a "rheumatoid character" of the disease. One reason for regarding these prolonged and intermittent cases of monarthritis as truly rheumatoid has been given by Sury (29) who pointed out the similar incidence of eye complications in these patients, as compared to those with a more typical polyarticular RA in children Morning stiffness is a typical finding in RA in adults and is also fairly common in children. The present material can not be used to estimate its incidence however as its importance has not been realized and notes on its occurrence were made only occasionally in the records. It is the author's impression that morning stiffness is not a rare finding in other types of arthritis in children but there is reason for indicating it as a *second* minor criterion. The *third* is the presence of subcutaneous nodules. They are not frequently encountered and may also occur in acute rheumatic fever The *fourth* is the exanthem variously described by different authors (13 16) as being a common phenomenon in childhood RA, especially among those running a more acute and septic course with high remittent fever Cases of this type were not seen in this material and no convincing notes describing this rash have been found. Gauchat & May (13) however observed it in 75 to 80 per cent of children with high fever in the initial stages. They described it as maculopapular in character and of a varying distribution similar in appearance to erythema multiforme and called it erythema multiforme rheumatoides. The *fifth* criterion are ocular manifestations, more precisely iridocyclitis, uveitis or band-shaped keratitis. It has been pointed out repeatedly (5 8 9 11 27 32) that these occur more often in children with RA than in adults which justifies their inclusion at least as a minor criterion. In the A.R.A. classification (6, 24) only

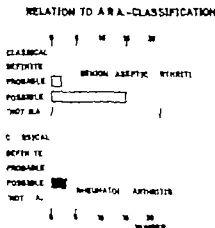


Fig. 6 Relation of the present diagnostic grouping to the ARA classification.

ritis is mentioned and for some reason only among the criteria for the more vague group "possible RA" Further extra-articular systemic manifestations of the rheumatoid disease can hardly be considered as typical or specific enough to be included among the diagnostic criteria As mentioned earlier for the purpose of this investigation the diagnosis of RA was made if at least two major or one major and two minor criteria were present.

In analogy with the A.R.A. classification a list of exclusions more adapted for pediatric use has been established. Some of them need comment There sometimes may be difficulties in the differential diagnosis of rheumatic fever which has given rise to the suggestion of special diagnostic criteria (19 23 31) Transitional forms between rheumatic fever and rheumatoid arthritis are known, but as a rule a distinction is possible and the different diseases run quite different courses the heart lesion being the decisive factor in the *former* and the joint affection in the latter In this connection fulfilled accepted criteria for rheumatic fever must be regarded as an exclusion factor for the diagnosis of RA. Joint symptoms are frequent in Henoch-Schoenlein purpura although admittedly rarely of a continuous duration of six weeks. Joint symptoms as an extraintestinal manifestation of ulcerative colitis have been observed to simulate the early stages of a RA and in one case they preceded the onset of the signs of colitis. A connection between urinary tract infection and polyarthritic symptoms has not been demonstrated in this material but on the other hand this connection has been well established in adults (7) and must be kept in mind also regarding children. The other exclusion factors are in concordance with some of those listed in the A.R.A classification.

It may be of interest to investigate how the diagnostic criteria presented here match the A.R.A. classification. This relation has been illustrated in Fig 6 Among those labelled as BAA two cases fulfil the qualifications for "probable RA In these two, symptoms were present from one knee joint for a little more than six weeks and from the other for about a month followed by a rapid recovery without recurrences, the observation time being three years in the first case and six years in the second. Of the remainder 15 children with *BAA satisfy the demands for* "possible RA" This is not surprising in view of the short duration of the joints symptoms being required for inclusion in the "*possible RA*" *group*.

Of the cases considered to have RA according to the diagnostic scheme presented here none fulfils the criteria for "classical RA" in conformity to the *A.R.A.* classification. This is partly due to the imperfect notes on the occurrence of morning

stiffness, the negative agglutination test in typical cases and the rarity of joint biopsies. As seen in Fig 6 the present classification in rigorousness lies between "definite" and "probable RA" Three of the RA cases have not fulfilled more criteria than corresponding to "possible RA In two of these there had been a long lasting, recurrent monarthritis, one of them presenting a residual lesion at the follow-up examination. In the third case which also ran a prolonged course, there was an interval exceeding three months between the two joints affected. There seems to be reason to regard these three cases as true RA.

The diagnostic scheme presented here being based on arbitrary conditions, shares the limitations of any attempt to construct diagnostic criteria in a similar way It has, on the other hand, proved to be of practical value in this investigation, giving prognostic information at a relatively early stage. It has thus been possible to make a definite classification in close to half of the cases (48 per cent) within two months after the first hospital admission.

The cases labelled a RA in th present series correspond essentially to the descriptions of the clinical picture and characteristics of typical juvenile rheumatoid arthritis. Heredity for rheumatic diseases in its widest meaning was present in 25 per cent. In Sury s material true RA was present in nine per cent among close relatives (29) Edstrom gave the figure of 23 per cent (8) Sairanen 30 per cent (25) Johnson & Dodd (17) stated that the incidence of a family history of rheumatic disease was possibly as high as 30 per cent. The preponderance for girls of the magnitude two to one has also been a constant finding in previous investigations. A peak incidence during the first three years of life has also been a regular observation. A preceding respiratory tract infection was found in the present material in 29 per cent Similar figures have been given in several studies, but Johnson & Dodd (17) quoted references establishing the incidence as high as 37.5 to 57 per cent and were of the opinion that an upper respiratory infection was seen prominently at some time during the course in about 70 per cent of the cases. Trauma close to the affected joint, within one month before the onset, occurred in eight per cent The analogous incidence given in earlier investigations has been of the same magnitude. A monarthritis is a common phenomenon early in the course An initial monarticular localization for at least one month was present in 29 per cent which is in concordance with corresponding figures given in the literature. In most of the present cases other joints were successively involved, but in six per cent of the whole material the disease remained monarticular There is also a close conformity as to previous reports, of the typical distribution of joints being affected, the knee joints being most commonly involved, followed by wrists, ankles and fingers. Heart disease was an uncommon finding in the present material, probably mainly due to the fact that the material does not contain patients with a septic course, which disposes to systemic involvement One further reason may be that ECG examinations have not been systematically made in each case In Sury s series (29) two children died of a rheumatoid heart disease and seven per cent showed signs of cardiac damage at

the follow up examination Edström (8) reported seven patients with Still s disease all of whom had cardiac involvement and 22 per cent of the others had signs of a heart disease. The frequency of eye lesions is of special interest Iritis and uveitis occurred in 12 per cent in this investigation Similar figures have been found by others (8 29) A permanent visual impairment is not rare as a sequelae

The concept that the prognosis in RA in children depends on how the material is selected seems justified. In a general pediatric ward a higher proportion of cases with a more limited course will be seen, whereas in a special institution for the treatment of RA, chronic and disabling cases will be overrepresented. The time of observation is of great importance. In the present investigation this comprises two to seven years which is too short for a definite evaluation, but is probably enough to provide an approximation of the probable course Of great importance also are the diagnostic criteria and if these are vague or too liberal it will be difficult to compare one clinic s experience with that of another Of the cases labelled as RA in this study most of them could be evaluated after three years course Fourteen per cent showed some degree of handicap This is a relatively favorable figure as is evident from the prognostic review given in the introduction. This is most likely due to the fact that the patients were derived from pediatric departments Evenso the prognosis in general is somewhat better in children than in adults

In the present investigation cases fulfilling the suggested criteria have been compared with another group indicated as benign aseptic arthritis There is no single symptom or finding placing a case in either category The most important differences apply to the age of onset and the course The RA cases commonly have their onset during the first five years of life; the BAA cases later The course of the latter is shorter and does not leave any functional limitation The preponderance for girls is the same in both groups and the distribution of joints being affected show similar trends although a monarticular course was more common among the BAA patients and the cervical spine was affected only in the RA cases. In both groups larger joints, especially the knees were most commonly involved early in the course Ocular manifestations favor the diagnosis of RA and was included among the criteria This was also the case concerning the positive agglutination reaction. Anemia was not uncommon among the RA children already within two months after the onset A preceding infection was a common finding in both groups and to label the BAA cases as postinfectious as a distinguishing feature does not seem justified.

The many similarities between the RA and BAA group inevitably raises the question of whether they do not merely represent different courses of the same disease This objection can hardly be contradicted, as long as the etiology of rheumatic diseases is unknown and no simple diagnostic test is available As pointed out conventional, serological methods to demonstrate the presence of the rheumatoid factor are unreliable in children. Awaiting further advances in this field the diagnosis of RA has to be based on a combination of different criteria. This is essential, if different materials are

to be compared as to the results of various therapeutic trials. In a pediatric hospital cases are frequently seen with arthritic symptoms in connection with other diseases. The classification elaborated by the A.R.A. is not applicable to children without modifications. The criteria suggested here have been useful from a practical point of view allowing a prognostic indication early in the course.

Summary

Diagnostic criteria for rheumatoid arthritis adapted for the features of the disease in children have been suggested and used for a classification in a follow-up examination. This study comprised 87 cases of aseptic arthritis of at least three weeks duration hospitalized in pediatric departments in Stockholm during 1952–1957. Those not fulfilling the criteria were labelled benign aseptic arthritis. They were compared with the rheumatoid arthritis cases as to the early clinical picture and the prognosis. The entirely different course in the two groups indicates that the suggested classification is of value in the prognostic prediction in cases of aseptic arthritis in children.

Acknowledgement

The valuable assistance of Mrs. Åsa Andersson and Miss Signe Wiberg is gratefully acknowledged.

References

1. Ansell, B. M. and Bywaters, E. G. L.: Growth in Still's disease. *Ann Rheum Dis* 15 295 1956.
2. — Prognosis in Still's disease. *Bull Rheum Dis* 9 189 1959.
3. Barkin, R. E.: The clinical course of juvenile rheumatoid arthritis. *Bull Rheum Dis*, 3 19 1952.
4. Bille, B.: Kronisk polyartrit hos barn och dess guldbehandling. *Nord Med*, 37 308, 1948.
5. Blegvad, O.: Iridocyclitis and disease of the joints in children. *Acta Opht* 19 219, 1941.
6. Diagnostic criteria for rheumatoid arthritis, 1958 revision by a committee of the American Rheumatism Association. *Ann Rheum Dis* 18 49 1959.
7. Dorfer, B., Gieseler, G., Ohlsson, B. and Romanus, R.: Genito-urinary focus in rheumatic disorder in the male. *Acta Chir Scand* 115 1 1958.
8. Edström, G.: Rheumatoid arthritis and Still's disease in children. A survey of 161 cases. *Arthritis Rheum*, 1 497 1958.
9. Edström, G. and Gedda, P. O.: Clinic and prognosis of rheumatoid arthritis in children. *Acta Rheum Scand* 3 129 1957.
10. Ellman, P. and Ball, R. E.: Rheumatoid disease with joint and pulmonary manifestations. *Brit Med J* 2 816, 1948.
11. Franceschetti, A. and Bamatter, F.: Augenveränderungen bei chronischer Polyarthritis im Kindesalter (Still'scher Formenkreis). *An Paediat*, 164 60, 1945.
12. Fyfe, W. M.: Rheumatoid arthritis in childhood. *Glasgow Med J* 36 102, 1955.
13. Goldhat, R. D. and May, C. D.: Early recognition of rheumatoid disease with comments on treatment. *Pediatrics*, 19 672, 1957.
14. Grokoest, A. W., Snyder, A. I. and Ragan, C.: Some aspects of rheumatoid arthritis. *Bull Rheum Dis*, 8 147 1957.
15. Isdale, I. C. and Bywaters, E. G. L.: The rash of rheumatoid arthritis and Still's disease. *Quart J Med*, 25 377 1956.
16. Johnson, R. E. and Hall, A. P.: Rubella arthritis. Report of cases studied by latex tests. *New Engl J Med*, 258 743, 1958.
17. Johnson, N. J. and Dodd, Katharine: Juvenile rheumatoid arthritis. *Med Cl N Amer* 39 459 1955.
18. Jones, T. D.: The diagnosis of rheumatic fever. JAMA, 126 481 1944.
19. Köttgen, U. and Callensee, W.: Statistische Untersuchungen zum kindlichen Rheumatismus. Der Rheumatismus, Vol. 34. Dr Dietrich Steinkopff Verlag, Darmstadt 1959.

20. McEwen C., Ziff M., Carmel, P DiTata, D and Tanner, M The relationship t rheumatoid arthritis of its so-called variants. *Arthritis Rheum, 1* 481 1958.
21 Norcross, B. M., Lockie, L. M. and Mc Leod C. C.: Juvenile rheumatoid arthritis. In Progress in Arthritis Ed. Talbott J H. and Lockie, L. M., Grune & Stratton, New York, 1958.
22. Pickard N S Rheumatoid arthritis in children. A clinical study *Arch Int Med, 80* 771 1947
23. Report by the rheumatic fever working party of the Medical Research Council of Great Britain and the American Heart Association: Treatment of acut rheumatic fever in children. *Brit Med J I* 555 1955.
24. Ropes, M. W Bennett G. A., Cobb S Jacox, R. and Jessar, R. A.: Proposed diagnostic criteria for rheumatoid arthritis. Report of a study conducted by a committee of American Rheumatism Association. *J Chron Dis 5* 630, 1957
25 Sairanen E On rheumatoid arthritis in children. A clinico-roentgenological study *Acta Rheum Scand Suppl. 2* 1958.
26. Schlesinger, B Rheumatoid arthritis in the young. *Brit Med J 2* 197 1949
27 Smiley W K., May E. and Bywaters, E. G L. Ocular presentations of Still' disease and their treatment. *A n Rheum Dis, 16* 371 1957
28 Still, G F.: On form of chronic joint disease in children. *Medicochirurgical transactions, 80* 47 1897
29 Sury B.: Rheumatoid Arthritis in Children. A Clinical Study Munksgaard, Copenhagen, 1952. Thesis, University of Copenhagen.
30. Tronut R.: La maladie de Still chez l'enfant. Thèse, Faculté de Médecine et de Pharmacie de Lyon, 1950.
31 Wallgren A.: The diagnosis of rheumatic fever *Ann Paediat Fenn, 3* 848, 1957
32. Vesterdal, E. and Sury B Indocyclitis and bandshaped corneal opacity in juvenile rheumatoid arthritis. *Acta Opht, 28* 322, 1950.
33. Wissler, H.: Der Rheumatismus im Kindesalter Teil 2: Die chronische Polyarthritis des Kindes. Verlag von Theodor Steinkopff, Dresden & Leipzig, 194..
34. *Wissler, H.: Subsepsis allergica. Helvet Paediat Acta, 13* 405, 1958.
35. Ziff M.: The agglutination reaction in rheumatoid arthritis. *J Chron Dis, 5* 644 1957
36. Ziff M., Brown P Lospalluto, J Badin J and M Ewen C.: Agglutination and inhibition by serum globulin in the sensitized sheep cell agglutination in rheumatoid arthritis. *Amer J Med, 20* 500, 1956

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BOOK REVIEWS

H Jones Reluctant Rebels. Re-education and Group Process in Residential Community

T vistock Publications, London 1960. 224 pages. Price 30s.

This book is based on an interesting pioneer experiment in residential therapy and re-education at "Woodmarch School a community of about 40 disturbed boys aged ten to fourteen years, whose difficulties had arisen mainly from unfavourable home circumstances. The staff members cooperated to establish a secure, orderly but tolerant atmosphere. The boys were permitted to operate a system of partial self-government through various committees. Principles f sociometry and group psychotherapy were used in the rehabilitation process. It was possible in this way to get a better under standing and a better control of the propor tion of social li that goes on beneath the surface of institutions of this kind. Lots of instructive examples are given from the everyday life at the school. Neighbourhood relations, staff problems and many other general problems usually connected with institutions of this kind are discussed. The book should be of great interest t everyone who works with institutions for mal-adjusted children.

Tom Reinand Goteborg

Edited by J Ch Siim. Human T x plasmosis. Munksgaard. Copenhagen, 1960. Price Dan. kr 48:—

A conference on clinical aspects and diag nostic problems f toxoplasmosis in pedia trics was held in Copenhagen at the VIII International Congress of Pediatrics in 1956. Last year the proceedings f this conference were reviewed and published in an elegant olume of 219 pages. In his introduction, A. Sabin stresses the importance of quantitatively estimating the role of toxoplasmosis as a cause of actual disease in contradistinction to the accumulated information that is currently vailable on the high incidence of clinically inapparent infections. In six studies on congenital toxoplasmosis (by M. Lelong F Bamatter H. Eichenwald and others) and in six on the acquired type (headed by the editor) the great variability of the clinical picture is amply exemplified. Differential diagnosis can be difficult, in clusion body disease can give the same clini cal picture as full-blown congenital toxoplasmosis and the acquired disease is seldom pathognomonic. Isolation of the organism by biopsy and animal inoculation is very important and is stressed by all contributors. Serological tests are specific but only signifi cantly rising titers indicat actual infection. (According t O Thalhammer a low dye test titer and a negative skin test indicate a recent infection.) The editor gives the following indications for testing serum for toxoplasma antibodies: fever of unknown origin, lymphadenopathy infectious mononucleosis (with negative Paul Bunnel test) relative lymphocytosis, encephalitis and serous meningitis, exanthema resembling typhoid rash, chorioretinitis and myocardi tis. Following broad indications for biopsy and serological testing Siim has been ble t show that 13 per cent of lymphadenopa thies f unknown origin were caused by toxoplasma. Two papers deal with ocular in olvement in toxoplasmosis, which may be a lat manifestation f the congenital form but is also seen in the acquired disease The treatment of toxoplasmosis is still in the tentative stage A combination f hemotherapy and cortisone has probably been of some value in the quired ocular form. Although the epidemiology is still obscure

it seems likely that animal toxoplasmosis is involved in the production of the disease in man Much more work has to be done before we can prevent this puzzling disease

Rutger Lagercrantz, Stockholm

Helen B Taussig Congenital Malformations of the Heart. Second edition. Vol. I, General considerations.

Harvard Univ Press, 1960

Volume I of the second edition of Dr Taussig book is designed for students and general practitioners It corresponds to approximately 90 pages of the 1947 edition. The plan of the new volume is basically the same All chapters have been revised, and a completely new 50-page part has been added on angiocardiography aortography and cardiac catheterization. In the embryology section, Streeter s horizontal concept is used. The etiologic and genetic aspects of congenital heart disease are more fully discussed, but the principles of analysis of malformations the changes in circulation at birth and the evaluation of the heart found at autopsy have not been brought up to date. In the chapter on methods of diagnosis, new material is added on aortic arch anomalies, the vascularity of the lungs, and the size of the pulmonary artery the section on ECG is extended. It is stressed that angiocardiography and catheterization are only aids in diagnosis, to be used t larify some specific point Further the dangers of these procedures are discussed at length. Comparatively little space is devoted to selective angiocardiography which is considered to give limited information and to be more dangerous. Less dilution of the dye and the risk of endocardial damage are mentioned as primary dangers. Many new illustrations ha e been added, mostly angiocardiograms which have been sharpened for didactic purposes.

Volume I of the revised ed t on of Dr Taussig work on congenital cardiac malformations represent a didactic survey for students and general pract tioners

B Ivemark Stockholm

Helen B Taussig Congenital Malformations of the Heart. Vol. II. Specific Malformations.

Harvard Univ Press, Cambridge 1960 1049 pp. Price $ 17 50

The second volume of the new edition of Dr Taussig s monograph corresponds to some 500 pages of the first edition. Consequently much new material is included, many new chapters have been added, and large parts have been extended. The basic plan of the old edition is somewhat hanged, the malformations being separated into four main groups, two according to the direction of the shunt and two dealing with the left and right side of the heart respectively As before, the treatise is focussed on the clinical picture with detailed information on physical findings with the addition of angiographic data. The illustrations comprise numerous radiographs, diagrams of the course of circulation and drawings of specimens. A new visual index gives a pictorial summary of signs and auscultatory fluoroscopic and electrocardiographic findings. This index enables the reader to grasp the clinical essentials of the various syndromes at a glance and is superior to the old ppendix correlating the diagnostic features.

New material in the group with venous-arterial shunt includes defective development of the right ventricle without VSD in which a discussion of Bernheim paper would perhaps have been interesting T this part is further added a valuable chapter on the Taussig Bing heart, and another on arteriovenous aneurysms. The complet transpositions are more fully treated and in the truncus chapter the hemitruncus concept is added.

Part two treats malformations of the right side of the heart. New material n this category includes multiple peripheral pulmonary stenoses and a 39-page section on pulmonary hypertension.

In the left-to-right shunt part one find a new section on aortic septal defect and completely rewritten chapter on abnormal pulmonary venous connection, including

drainage into the inferior vena cava with sequestered lung ("scimitar syndrome") The treatise on ASD now includes the atrio-ventricular defect

In the fourth part new sections are found on vascular rings, coronary arteriovenous fistulae fibroelastosis, glycogenosis, cor triatriatum and corrected transposition. The reviewer might miss the clinical correlations with detailed anatomical findings, but Dr Taussig's monograph should still be a mine of information to cardiologists and pediatricians.

G Ivemark, Stockholm

Dr Med. Paul Heintzen. Quantitative Phonokardiographie am Beispiel der respiratorischen und pressorischen Schwankungen der Herztätigkeit Georg Thieme Verlag, Stuttgart 1961 187 pages, 101 figures. DM 28.—

After general discussion of the origin of different heart sounds, the author reports his own results from study of the influence of respiration upon the phonocardiographic recording of the heart sounds. The effect of respiration upon hemodynamic conditions and the relation between phonocardiographic and hemodynamic changes during normal respiration and valsalva tests are discussed. A very complete bibliography on the subject is added.

B Strandberg Stockholm

Th. Hellbrügge, J Rutenfranz and O Graf Gesundheit und Leistungsfähigkeit im Kindes- und Jugendalter.

Georg Thieme Verlag, Stuttgart, 1960. 293 pages. Price DM 34.—

This monograph deals with certain problems of health and physical fitness in pediatrics. The 480 references give good review especially of the German literature in this field. Several parts of the book are well known to the pediatrician, for instance the introductory chapters regarding mortality morbidity growth and development. Of greater interest are the parts that describe physical working capacity in different ages and its dependence upon hemodynamic, respiratory and muscular factors. Of value also is the account of various methods for measuring the working capacity of an individual, in which the authors quote their own studies of the so-called "Leistungspulsindex" in a series of 940 subjects aged 8–25 years. In a chapter on recovery after work the pulse restitution is discussed in relation to the length of the anaerobic phase at the start of the test. The younger the individual the shorter is this phase and the quicker does the restitution after the termination of the work occur The working and leisure conditions in school-children are compared with those in working adolescents. The authors review the degree in which national and international legislation and practice have taken into account the special physiology of the ages of growth.

Yngve Larsson, Stockholm

F Bertram and G Michael Internationales Biguanid Symposium am 1... und 13 Mai 1960 in Aachen.

Georg Thieme Verlag, Stuttgart, 1960 167 pp. Price DM 16.80.

The place of the sulfonyl-urea drugs in the treatment of diabetes is well established. As regards the more recently introduced biguanides the situation is more doubtful. The Aachen symposium in 1960 was arranged in order to throw more light on these substances. Among available biguanides the phenylaethyl compound, phenformin, is the most commonly used. In contrast to the sulfonyl ureas its mode of action is extrapancreatic, otherwise unknown. The theory of stimulation of intracellular anaerobic glycolysis has not been satisfactorily proved, nor the related hypothesis of inhibition of respiratory enzymes. Of interest is the increase in lactat and pyruvat in blood serum during treatment with phenformin. In some cases a tendency to normoglycemic ketosis may make interruption of treatment necessary The clinical experiences of the effect of phenformin and related biguanides are not unanimous, probably dependent on differences in the selection of cases for treatment. In older patients about 40–60 per cent seem to respond to the drug in juvenile cases no more

than about one third. The experience of a pediatric clinic is reported by Rosenkranz, who found satisfactory results in 7 of 17 patients. This meant lower insulin requirement and stabilization of labile cases. Combined treatment with sulfonyl-ureas may in some patients be valuable. Gastrointestinal side effects may occur but do not seem to be a serious problem. One case of severe thrombopenia was reported by Koopmann, otherwise there have been no blood dyscrasias

Yngve Larsson Stockholm

F Gegesi Kiss and Gy Szutrély Herz und Kreislaufstörungen im Säuglings- und Kindesalter

Verlag der Ungarischen Akademie der Wissenschaften, Budapest, 1960 880 pages, 7 figures.

This book is the German translation of the second edition of a Hungarian publication which appeared in 1953 According to the preface it is especially written for pediatricians and general practitioners. The authors base their statements on personal clinical experience their own research work and current literature and especially quote their own publications, while most of the contributions by eminent foreign cardiologists such as Keith Leatham, Nadas and Wood are overlooked The very detailed theoretical discussion of certain concepts, for instance, cell-structure and metabolism of the myocardium, as well as diabetes mellitus, hypoglycemia and serum-disease are given too much space for a book meant for general practitioners and pediatricians. The congenital malformations of the heart, on the other hand, are *too superficially treated*. This chapter even from a factual point of view is not always beyond criticism. The statement that the growth in height is retarded in children with patent ductus arteriosus is too categoric and pertains only to more severe cases. Their opinion that bacterial endocarditis occurs very often in association with atrial septal defect is not in agreement with most authors. Among the diagnostic procedures the very valuable *selective angiocardiography according to Jönsson* and coworkers is not mentioned The normal blood pressure in the pulmonary artery is given as 40/10 mm Hg which seems to be rather high. With regard to rheumatic fever the reviewer will only mention that the authors are of the opinion that the causative agent is a virus and that acute pericarditis often leads to constriction, a concept with which few authors will agree The therapy of cardiac and circulatory failure is *treated rather thoroughly and* reflects the vast experience of the authors. The recommendations of restrictions with regard to sport and exercise after acute infectious diseases and cardiac operations seem rather severe The book is not very attractive from typographical point of view and is not easy to read. The figures are however rather good and satisfactorily instructive even if not of the highest class.

L. E Carlgren Gothenburg

ANNOUNCEMENT

The Department of Pediatrics of the University of Buffalo School of Medicine has established a *research training program for persons planning a career in pediatric research and teaching* Programs will be planned for each individual trainee for a period of at least two years with at least one year spent in study or research in a basic science department of the University of Buffalo School of Medicine followed by a year of research within the Department of Pediatrics. Qualifications include a minimum of one year pediatric residency. Stipends are $6000 first year $6500 second year plus $500 per dependent up to three dependents. Funds are available for laboratory expenses and travel. Inquiries may be sent to Dr Robert Guthrie Children's Hospital 19 Bryant Street Buffalo 22, New York.

Acta Pædiatrica 50 549–560 Nov 1961

From the Department of Metabolic Research (Head: Dr Harry Boström) Wenner-Gren Institute, University of Stockholm, and the Pediatric Clinic (Head: Prof John Lind) Karolinska Sjukhuset Stockholm

Studies on Ester Sulphates

12 Excretion of Sulphate Acceptors in Phenylketonuria

by HARRY BOSTRÖM and BO WENGLE

It is an established fact that many phenolic, indolic and steroid compounds are excreted in the urine of mammals. Before excretion most of these compounds are converted to some extent into esters of either glucuronic or sulphuric acid. The enzymatic mechanism responsible for sulphate and glucuronide conjugation is nowadays known in some detail.

Sulphate conjugation has been shown to be a two-step reaction involving sulphate activation and sulphate transfer (12, 18) and the enzymes catalyzing these reactions are present in many tissues, e.g. liver, intestinal mucosa, cartilage and cornea. The enzymes involved in sulphate conjugation are water-soluble and particle-free extracts containing sulphate-activating and sulphate-transferring enzymes have been prepared from various tissues. When a sulphate acceptor, e.g. a phenol or a steroid, is incubated in a medium containing an active tissue extract, ATP, inorganic sulphate and magnesium ions in a suitable buffer solution, the corresponding ester sulphate is formed in vitro (11, 16, 17, 22). By adding S^{35}-labelled sulphate to such a system, the ester sulphate formed can easily be demonstrated by means of paper-chromatographic and electrophoretic methods, combined with autoradiography.

On the basis of these principles, a method was recently worked out in our laboratory for the demonstration of sulphate acceptors in human urine under normal and pathologic conditions (9). In the present investigation, this technique was applied in phenylketonuria—a condition known to be associated with characteristic disturbances in the metabolism of aromatic amino acids.

The main feature of this inborn error of metabolism is probably a defect in the enzyme system normally hydroxylating phenylalanine to tyrosine (13), which results in a raised excretion of phenylalanine (PA) and its derivatives phenylpyruvic acid (PPA), β-phenyl-L-lactic acid (PLA) and phenylacetic acid (PAA), the last mentioned chiefly in the form of phenylacetyl glutamine (PAG) (14, 23).

In addition to the metabolites mentioned, some phenolic acids are excreted in

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abnormal amounts. Thus the metabolites of tyrosine p-hydroxyphenylpyruvic acid (p-HPPA), p-hydroxyphenyllactic acid (p-HPLA) and p-hydroxyphenylacetic acid (p-HPAA) are said to be excreted in raised quantities as determined by paper chromatography (1, 7, 8, 21).

The most striking difference between the metabolism of phenolic acids in normal subjects and in phenylketonurics is, however, the hundredfold increased excretion of o-hydroxyphenylacetic acid (o-HPAA) in the urine of the latter (3). Although the mechanism of formation of this phenolic acid is uncertain, its precursor seems likely to be phenylpyruvic acid and not o-tyrosine, which has not yet been recovered from human urine (10).

A raised excretion of indolyllactic and indolylacetic acids has also been demonstrated in phenylketonuria, thus indicating a deranged metabolism of tryptophan (2).

In 1951 Bickel introduced a low phenylalanine diet in the therapy of affected children (5). It was found to normalize the excretion of phenylalanine and its metabolites, as well as that of o-HPAA and indolic compounds (3, 5, 15, 14). The excretion of tyrosine metabolites was, however, said to remain unaltered (7, 8).

In the present investigation, a comparison was made between the urinary sulphate-acceptor pattern of normal subjects and phenylketonurics. It disclosed that when urine specimens from phenylketonurics were incubated in the sulphurylating system, at least two conjugates were formed that could not be detected in normal urines treated in the same way. These findings will be reported and discussed in the following.

Methods and Material

Sulphate-conjugation technique

100 µl of a carrier free solution of S35-labelled sulphate* (10 mC/ml) were evaporated to dryness in each of a series of test tubes. The test tubes were then placed in an ice-bath, and the following solutions added to each tube:

a. 50 µl of a solution containing equal parts of 0.3 *M* potassium phosphate buffer pH 6.8, 0.03 *M* potassium sulphate and 0.005 *M* magnesium chloride.

b. 10 µl of 0.04 *M* ATP.

c. 40 µl of a particle free supernatant of rat-liver homogenate prepared according to Roy (16).

d. 10 µl *of human urine. If the urine was pretreated and extracted with ether, a portion of the extract corresponding to 50 µl of urine was evaporated to dryness in the test tube before the* S35-labelled sulphate was added.

In each experimental series, two types of control experiment were run:

A. Complete system with enzyme solution and urine replaced by water.

B. Complete system with urine specimens replaced by water.

All test tubes containing the components listed above were then incubated in a water bath at 37°C for 3 hrs.

Demonstration of ester sulphates

Five µl samples of the incubated mixtures were subjected to paper electrophoresis and two-dimensional ascending paper chromatography on Whatman No. 1 filter paper. The following solvents were used:

I. *Phenol-water* (400 g/100 g). A beaker containing concentrated aqueous ammonia was placed in the chromatographic tank.

II. The upper phase of n-butanol-2 N ammonia (1/1 by vol.).

III. n-butanol-acetic acid-water (1/3/5 by vol.)

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* Obtained from the Radiochemical Centre, Amersham, England.

Of these three solvents, no I was combi ned with either II or III. Solvent no. I was always used as first solvent

Paper electrophoresis was performed in 0.075 M acetate buffer (pH 5.5) for 1 hr at 30 or 40 V/cm.

The dried electropherograms and chroma tograms were subjected t utoradiography on Gevaert Curix X-ray film, and exposed for one week. Gevaert G 150 was used as developer

Pretreatment of urine

Addition of more than 10 µl of urine to the enzyme system produced some inhibition of the sulphurylating activity W therefore chose in some experiments to concentrat the urine specimens by freeze-drying and extracting the dry residue with ether t take up aromatic and other organic compounds. These extracts were then free from salts, and could be added in amounts cor responding t 50–100 µl of urine without inhibiting the system. In addition t untreated urine specimens, two types of urinary ex tract were incubated with the sulphurylating system, after evaporation f the organic solvent

1. Ether extract of a freeze-dried urine specimen.

2. Ether extract of a neutralized and freeze-dried urine specimen, previously hyd rolyzed in 1 N HCl for 60 minutes at 100°C

All urines tested in the enzyme system were night specimens collected without the addition f any preservative. All subjects had been fasting since 8 p.m. on the preceding day The samples were brought to the laboratory on the same day they were voided, and the experiments were started within a few hours of thei arrival. Otherwise, the urine specimens were kept in deep-frozen condition until used.

Chromatography of urinary am no acids

Desalted urines were subjected t two-dimensional ascending paper chromatography on Whatman No. 1 paper in the frames described by Datta, Dent & Harris (10). The following solvents were used:

I n butanol-acetic acid water (L./3/5 by vol.).

II Phenol-water-aqueous ammonia (400 g/100 g/2.5 ml)

The chromatograms were developed by dipping them into 0.. per cent ninhydrin in acetone

Determination of phenylalanine n blood

Determination of PA in blood specimens was performed by the method of Berry (4)

Est mation of o-HPAA

o-HPAA in urine specimens was estimated as follows Urinary aliquots were chromatographed directly in amounts ranging from 20 to 100 µl, depending on the amount of o-HPAA present. The solvent system used was *iso* propyl alcohol—aqueous ammonia—water (*0/1/2 by vol.) descending chromatography on Whatman No. 1 paper The chromatograms were developed by spraying the papers with a dilut ethanolic solution of 2,6-dibromoquinone chlorimide followed by queous ammonia. The intensity of the blue staining was compared with the colour of known amounts of o-HPAA chromatographed on the same paper This spraying reagent was found to be more sensitive and specific to o-HPAA than diazotized sulph anilic acid, and made it unnecessary to ex tract the substance from the urines before the chromatograms were run. Recovery f added amount of o-HPAA to urine speci mens was in the range of ±*0%.

Preparation of reference substances

The S^{35}-labelled ester sulphates of o- and p-HPAA were prepared from commercial preparations f the latter by enzymatic sulphurylation *in vitro* in the rat liver super natant system, followed by isolation of the relevant esters by paper electrophoresis (autoradiographic indication) and subsequent lution and freeze-drying The labelled material was stored t 20°C.

The identity of the sulphate ester f o-HPAA formed was checked by identification

Obtained from L. Light & Co., Colnbrook, Bucks., England.

of the split products, after acid hydrolysis in 1 N HCl for 1 hr. The liberated sulphate was shown to migrate in high voltage isotrophoresis at the same rate as a specimen of S35-labelled sulphate, and o-HPAA was identified in the aforementioned system for estimation of o-HPAA.

The ester sulphate of 5-(p-hydroxybenzyl)-hydantoin was prepared according to the same principle from a specimen of the hydantoin obtained by synthesis from p-HPPA and urea in the presence of hydrochloric acid (6).

In addition to these reference substances, several other ester sulphates of steroids, phenols and indoles have previously been produced by enzymatic sulphurylation in our laboratory and their chromatographic and electrophoretic data have been established.

Determination of creatinine

Creatinine was determined by a modified Jaffe procedure (20).

Case Material

Eight cases of phenylketonuria were studied. In every case, the diagnosis was established biochemically by demonstration of pathologically raised urinary excretion of PA, PPA and o-HPAA, and a high PA level in the blood. A summary of the relevant laboratory data is given in Table I. Cases 1–4 had not been given a low PA diet. In Cases 5–8, this diet was tested with a varying degree of success. Urinary studies both before and during treatment could therefore be made in the latter group.

Case 1. A girl b. 1942, sib of Case 4. She was severely mentally retarded, and was still unable to talk. Retarded development was first observed at 8 months of age.

Case 2. A boy b. 1943. His I.Q. had been 30 since the first test at 8 years of age.

Case 3. A boy twin brother of Case 2. Anthropologic studies, including blood typing, suggested monozygosity. His I.Q. had been 50 since the first test at 8 years of age. Retarded development was first observed in both twins at 6 months.

Obtained from Dr. Theodor Schuchardt, GMBH & Co., München, Germany

Case 4. A boy b. 1944, sib of Case 1. He was severely mentally retarded, and was still unable to talk. Mental retardation was first noticed when he was 6 months old.

Case 5. A boy b. April 15, 1936. Retarded development was first observed at 6 months of age. He had seizures, and his EEG was pathologic. Since Oct. 4, 1957 he had been given a low PA diet. No marked mental improvement could be demonstrated after 2½ years treatment. On the other hand, he ceased to have seizures, and his EEG became normal. In addition, his contact with the nursing staff improved considerably during treatment.

Case 6. A girl b. Dec. 23, 1957. Her development was apparently normal up to at least 1 year of age. She sat without support at 7 months, and could say a few simple words before she was 1 year. When she was 1 year old, her mother noticed for the first time that the patient's urine had a peculiar smell. At 13 months, she started to walk, although somewhat unsteadily. One month later she stopped walking, became less active, and seemed drowsy and tired. The diagnosis of phenylketonuria was confirmed at 18 months of age, and a low PA diet was instituted. From being anxious and peevish, she became considerably more cheerful and active. At 20 months, she started to walk again, and to say a few words. Psychologic tests at 23 months of age showed somewhat retarded development, chiefly with respect to vocabulary.

Case 7. A boy b. May *2, 1958. He was hospitalized at the age of 8½ months, on account of retarded development. He was unable to sit, and balanced his head unsteadily. He had seizures, and his EEG was pathologic. A low PA diet was started at 9½ months. This resulted in a considerable improvement in his condition including the EEG. His seizures stopped. His contact with his environment became much better and he learnt to sit fairly steadily. He was able to stand without support before 17 months.

Case 8. A girl, b. Aug. 28, 1958. Asthmatic complaints started when she was a few

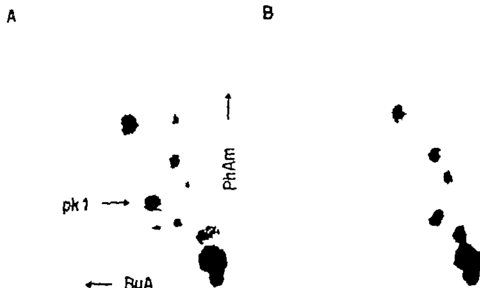


Fig. 1 Chromato-autoradiograms showing the "ester-sulphate pattern" of urine specimens not pretreated before incubation with the sulphurylating system. A. Urinary pattern of one untreated case of phenylketonuria (Case 6). B. Urinary pattern of one normal subject of the same age and sex as Case 6. The position of pk 1 as indicated on chromatogram A, corresponds to an empty area on chromatogram B (indicated by the dotted ring). PhAm and BuA represent solvents I and III, respectively (cf. text)

months old. Her urine had a strong, peculiar smell since the age of 4 months. She was unable to sit without support at 11 months when a low PA diet was instituted. At 16 months she could sit unsupported, but could not stand, even with support. Psychologic tests at 12 and 16 months showed no appreciable mental improvement. Her development seemed to be somewhat below the normal for a child half her age.

The controls consisted of 8 parents and 3 sibs of phenylketonurics, in addition to 20 healthy subjects, aged 3–63 years (members of the laboratory staff and their relatives) with no known history of phenylketonuria in their families.

Results

Ester-sulphate pattern of normals

Incubation of normal urine with the aforementioned sulphate-conjugating technique resulted in the formation of about 25 S^{35} labelled ester sulphates. They produced a characteristic pattern of spots on paper chromatography or on paper electrophoresis in combination with autoradiography. Although this "ester-sulphate pattern" varied from case to case its main features seemed to be constant (Fig. 1 B).

Incubation of ether extracts of normal urine specimens subjected to acid hydrolysis revealed a pattern differing in several respects from the original one. It contained more spots (ester sulphates) than originally present. On the other hand, certain ester sulphates formed when untreated urine specimens were incubated with the sulphurylating system could no longer be detected (Fig. 2 D).

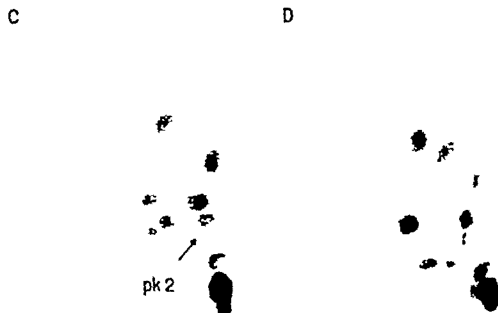


Fig — Chromato-autoradiograms showing the ester-sulphat pattern of urine specimens *acidly hydrolyzed* before incubation with the sulphurylating system. C Urinary pattern of one untreated case of phenylketonuria (Case 8). D Urinary pattern of the same normal subject as in Fig. 1B. The position of pk —, as indicated on chromatogram C, corresponds to an empty res on chromatogram D (indicated by the dotted ring). Sol ent systems same as in Fig 1

Ester-sulphate pattern of untreated phenylketonurics

Urine specimens of seven untreated phenylketonuric patients all excreting pathologically raised amounts of PPA and o-HPAA (Table 1) were sulphurylated as described The sulphate acceptors of these pathologic specimens formed ester sulphates which produced patterns deviating characteristically and uniformly from those in normal subject

The pattern of urine specimens that were not pretreated showed in every case a new distinct spot pk 1 n the autoradiograms (Figs 1 A, 3 and 4)

The ester-sulphat pattern of acidly hydrolyzed phenylketonuric specimens revealed further peculiarities In addition to pk 1 another ester sulphate appeared, not present in normal patterns This ester sulphate (pk 2) gave a well marked spot on the chromato-autoradiograms when running the chromatograms in solvents I and III (Fig 2 C) On the electropherograms pk 2 moved relatively slowly toward the anode which distinguished it fairly well from other labelled ester sulphates (Fig 4)

Ester-sulphate pattern of phenylketonurics given a low phenylalanine diet

Three phenylketonuric patient (Cases 5 6 and 8) were considered to be adequately treated by a low phenylalanine diet in view of our biochemical findings (normal phenylalanine level in blood, nor

TABLE 1 *Summary of laboratory data in the 8 cases of phenylketonuria studied in the present paper*

Case No.	Duration of treat ment mths	Blood PA mg/100 ml	$FeCl_3$ and 2,4-dinitro-phenylhydrazine test	Excretion of PA	Approx. amount of µg o-HPAA/mg creatinine	pk 1	pk 2
1	0	33	+	+	170	+	+
2	0	25	+	+	180	+	+
3	0	20	+	+	100	+	+
4	0	40	+	+	110	+	+
5	0	30–40–60	+	+	pathol. amounts	?	?
5	29	< 2.5	—	—	< 5	—	traces
6	0	20	+	+	250	+	+
6	3½	< 2.5	—	—	< 5	—	+
7	0	25	+	+	73	+	+
	13	?	traces	+	20	(+)	+
8	0	45	+	+	190	+	+
8	6	< 2.5	—	—	10	—	+

A fresh aqueous solution of $FeCl_3$ and saturated solution of 2,4-dinitro-phenylhydrazine in 2 N HCl were used as tests for the presence of PPA.

mal excretion of phenylalanine, PPA and o-HPAA. Table 1) Their urine specimens contained no sulphate acceptors able to form any detectable abnormal ester sulphates when incubated unhydrolyzed in the sulphurylating system. Thus, pk 1 was lacking in the pattern. On the other hand, hydrolysis of the specimens showed that the sulphate acceptor giving rise to pk 2 was still excreted by two of the patients (Cases 6 and 8) despite several months duration of treatment. The precursor of pk seemed, in fact to be excreted in the same amounts during treatment as before any dietary regime was started.

Urine specimens of the remaining patient (Case 5)—who had been treated for the longest period—gave rise to barely detectable amounts of pk 2, indicating that the excretion of its precursor had been practically normalized.

Ester-sulphate pattern of relatives of phenylketonurics

The urinary sulphate acceptors of eight parents (heterozygotes) and three unaffected sibs of phenylketonurics were also studied by the sulphate-conjugating technique. Neither pk 1 nor pk 2 could be detected in the "ester-sulphat pattern" of these persons, which therefore did not differ in any way from the normal pattern.

Identification of pk 1

The position of pk 1 on paper chromatograms (Fig 1 A) and electropherograms (Figs. 3 and 4) was found to be characteristic for the ester sulphate of o-HPAA. Cross-matching experiments with reference substances in three independent chromatographic solvent systems (solvents I, II and III) and in one electrophoretic system (0.075 M acetate buffer

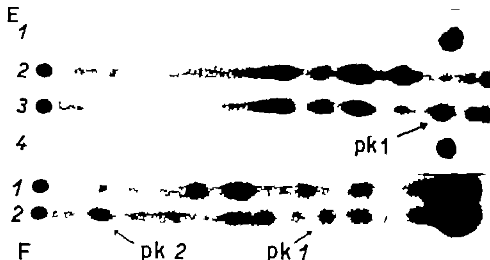


Fig. 3. Electrophero-autoradiogram showing the difference between the urinary ester-sulphate pattern" of one normal (E 2) and one phenylketonuric boy (E 3). The urine specimens were not pretreated before incubation with the sulphurylating system. Starting points to the left in the picture. E 1 and 4 biosynthetically formed samples of the ester sulphate of o-HPAA. The position of pk 1 is indicated in the figure. Electrophoresis was performed in 0.075 M acetate buffer pH 5.5 (40 V/cm).

Fig. 4. Electrophero-autoradiogram showing the patterns of the ester sulphates studied by chromatography in Fig. 2. Starting points to the left in the figure. F 1 corresponds to Fig. 2 D (normal). F 2 corresponds to Fig. 2 C (phenylketonuric). PK 1 and pk 2 are indicated in the figure. Electrophoresis was performed in 0.075 M acetate buffer pH 5.5 (30V/cm).

pH 5.5 30–40 V/cm) also indicated that pk 1 was identical with the ester sulphate of o-HPAA

Identification of pk 2

It could further be shown that none of the hitherto known abnormal metabolites excreted in phenylketonuria could give rise to an ester sulphate with the properties of pk 2, when incubated in the sulphurylating system. In extraction experiments, it was found that the sulphate acceptor giving pk 2 could not be extracted with ether or ethyl acetate from the acidly hydrolyzed urines until they had been made alkaline with sodium bicarbonate. If prior to hydrolysis, the urines were acidly extracted with ether or ethyl acetate, pk 2 could not be detected after incubation of the acidly hydrolyzed residues.

A mixture of p-HPPA and urea was hydrolyzed in the same way as urine and incubated with the sulphurylating system. The hydantoin formed gave rise to an ester sulphate having the same properties as pk 2 on both chromatograms and electropherograms. Similar treatment of normal urines, after addition of certain amounts of p-HPPA also revealed a labelled spot with the same characteristics as pk 2 on the autoradiograms of paper chromatograms and electropherograms.

During the hydrolysis of urine specimens of phenylketonurics, a compound was formed, which evidently consisted of 5-

(*p*-hydroxybenzyl)-hydantoin, its sulphuric acid ester being subsequently visualized on the autoradiograms as pk ...

Discussion

The enzymatic technique for demonstration of urinary sulphate acceptors worked out at this laboratory seems to possess high sensitivity Thus detailed ester sulphate patterns containing about 25 compounds or more were revealed when 5 µl samples of the incubating media (corresponding to 0.5 µl of urine) were applied to chromato-autoradiography An increase in the sensitivity and specificity of the method was obtained by the slight modification introduced in the present study *i.e.*, extraction of the urines before incubation. Another way of enhancing the possibility of detecting small amounts of certain compounds would be to raise the quantity of S^{35}-labelled sulphate added, or to expose the X ray films for a longer period than one week.

Various solvents can be used for paper chromatographic separation of the sulphated compounds In the present study phenol water—which gave good spreading of the compounds—was always used as first solvent. n-butanol-acetic acid water (III) was preferred as second solvent, because ester sulphates of phenoll acids of interest in phenylketonuria were much better separated in this system than in solvent II. Other solvent combinations might be more useful for the separation of other types of esterified compounds.

Hydrolysis of the urines prior to incubation increased the number of spots, and the overall pattern was greatly strengthened. A change in ppearance of the patterns occurred after hydrolysis, due to new compounds being available for sulphurylation, after splitting of acid-labile conjugates. In addition, certain compounds were evidently destroyed during acid treatment since the patterns of untreated urines contained some ester sulphates that could not be detected after corresponding treatment of hydrolyzed urines.

The excretion of *o*-HPAA previously observed by other authors (3 7) could easily be demonstrated by the present technique, since the most striking difference between the normal and phenylketonuric patterns was the appearance of the ester sulphate of this compound (pk 1) In agreement with earlier findings (3 7) it could also be shown that the excretion of *o*-HPAA was normalized by a low phenylalanine diet

The studies on hydrolyzed urines from the patients did not disclose the excretion of any conjugated, pathologic sulphate acceptors. The new spot, pk 2—which actually appeared after the sulphurylation of hydrolyzed urines—was most probably identical with the ester sulphate of 5-(*p*-hydroxybenzyl)-hydantoin, a condensation product of *p*-HPPA and urea. It seems likely that the amount of pk 2 formed was correlated to the amounts of *p*-HPPA excreted, since the urinary urea concentration is certainly in excess for this condensation reaction.

Urine specimens of seven phenylketonurics gave rise to pk 2, despite the fact that some of them had been treated for a long period (2½–13 months). Pk 2 was however barely detectable in the ester sulphate patterns of hydrolyzed urine specimens of Case 5 (treated for 9 months) indicating an almost normalized

excretion of p-HPPA The difference between the strength of pk 2 in these patterns and in those of the other patients was great. Unfortunately no data are available on the excretion of p-HPPA in Case 5 before a low phenylalanine diet was instituted. There is, however no reason to believe that the excretion of p-HPPA had been normal before treatment since both the present study and earlier ones have shown that pathologic amounts of this acid are excreted in phenylketonuria

It has also been claimed that the excretion of p-hydroxyphenyl derivatives remains unchanged during treatment of patients with a low phenylalanine diet (7 8) The patient in question, who had been given this diet for 29 months, nevertheless excreted only small amounts of p-HPPA, which might indicate a slow normalization in the degradation of phenolic acids derived from tyrosine

Of the cases of phenylketonuria discussed in this paper Case 6 was the most remarkable from several aspects.—Firstly this patient s development was obviously normal until at least one year of age thus showing an unusually long lag period before symptoms of the disease appeared. Secondly a rapid loss of already acquired abilities (e.g walking and saying a few words) occurred at the age of 13–14 months. Thirdly a low phenylalanine diet which was not started until the age of 18 months nevertheless restored the aforementioned abilities in a very short time.—The slow development of phenylketonuria could also be correlated to the biochemical findings in this patient who most often showed comparatively low phenylalanine figures in blood, and sometimes even negative or only slightly positive tests for PPA in urine It seems reasonable to suggest that this case represents a mild form of phenylketonuria. Moreover it seems to demonstrate the value of dietary therapy despite its late institution. The observations made in this patient thus seem to support the view that the critical factor in the dietary treatment of phenylketonuria is not only the age of the patient at the onset of treatment, but the actual duration of the manifestations as well.

The enzymatic technique applied in the present study of phenylketonuria did not reveal any previously unknown metabolite On the other hand, the results obtained were in good agreement with our present knowledge of this disease Consequently it is suggested that this technique could be of value in the search for unknown sulphate acceptors in less well characterized metabolic diseases.

Summary

1 The pattern of sulphate acceptors present in the urine of phenylketonurics and control cases was visualized by chromato-autoradiographic methods, after treatment of urine specimens in a sulphurylating rat-liver system in the presence of S^{35} labelled sulphate

2. The sulphate of o-HPAA formed during this procedure from o-HPAA excreted in the urine of phenylketonurics could be demonstrated as a well-defined spot (pk 1) on the autoradiogram in all untreated cases.

3 Another sulphate acceptor revealed after acid hydrolysis was shown to be identical with the hydantoin formed from urea and p-HPPA during hydrolysis. The

concentration of the ester sulphate of this hydantoin (pk 2) probably reflects the amount of p-HPPA excreted in phenylketonuria. This compound did not vanish from the urine specimens of patients given a low phenylalanine diet for periods of up to 13 months. It was however absent in one case treated for 29 months. The significance of these findings, as well as the possibilities of the method applied, are discussed.

Acknowledgements

The authors' thanks are due to Professor J. Lind for his interest in our work, and for permission to perform this study in cases of phenylketonuria attending the Pediatric department, Karolinska Sjukhuset, Stockholm, as well as to Drs. E. Jacobsson, K. M. Herrlin and A. Alvin, who kindly placed their cases at our disposal.

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References

1. Armstrong, M. D. and Robinson, K. S.: Abnormal metabolites in phenylketonuria. *Fed Proc*, *13* 175, 1954.
2. — On the excretion of indole derivatives in phenylketonuria. *Arch Biochem*, *52* 287 1954.
3. Armstrong, M. D., Shaw, K. N. F. and Robinson, K. S.: Studies on phenylketonuria. II. The excretion of o-hydroxyphenylacetic acid in phenylketonuria. *J Biol Chem*, *212* 797 1955.
4. Berry, H. K.: Paper chromatographic method for estimation of phenylalanine. *Proc Soc Exp Biol* (N. Y.), *95* 71 1957.
5. Bickel, H., Gerrard, J. and Hickmans, E. M.: The influence of phenylalanine intake on the chemistry and behaviour of a phenylketonuric child. *Acta Paediat*, *43* 64, 1954.
6. Boscott, R. J.: Unpublished observation. (Quoted by Boscott, R. J. and Cooke, W. T.: Ascorbic acid requirements and urinary excretion of p-hydroxyphenylacetic acid in steatorrhoea and macrocytic anaemia. *Quart J Med*, *23* 307 1954.
7. Boscott, R. J. and Bickel, H.: Detection of some new abnormal metabolites in the urine of phenylketonuria. *Scand J Clin Lab Invest*, *5* 380, 1953.
8. — Phenylalanine and tyrosine metabolism in patients with phenylketonuria. *Biochem J* *56* I 1954.
9. Boström, H. and Vestermark, A.: Studies on ester sulphates. 9. On the occurrence of sulphate acceptors in human urine. *Scand J Clin Lab Invest*, *12* 323. 1960.
10. Datta, S. P., Dent, C. E. and Harris, H.: An apparatus for the simultaneous production of many two-dimensional paper chromatograms. *Science*, *112* 621 1950.
11. De Meio, R. H., Wizerkaniuk, M. and Fabiani, E.: Rôle of adenosinetriphosphate in the enzymatic synthesis of phenyl sulphate. *J Biol Chem*, *203* 257 1953.
12. De Meio, R. H., Wizerkaniuk, M. and Schreibman, I.: Enzymatic system synthesizing sulphuric acid esters of phenols. *J Biol Chem*, *213* 439 1955.
13. Jervis, G. A.: Studies on phenylpyruvic oligophrenia. The position of the metabolic error. *J Biol Chem*, *169* 651 1947.
14. — Excretion of phenylalanine and derivatives in phenylpyruvic oligophrenia. *Proc Soc Exp Biol* (N. Y.), *75* 83, 1950.
15. Knox, W. E. and Edwards, S. W.: Unpublished observation. (Quoted by Knox, W. E. and Hsia, D. Y. Y.: Pathogenetic problems in phenylketonuria. *Amer J Med* *22* 687 1957.)
16. Roy, A. B.: The enzymatic synthesis of steroid sulphates. *Biochem J* *63* 294, 1956.
17. Schneider, J. J. and Lewbart, M. L.: Enzymatic synthesis of steroid sulphates. *J Biol Chem*, *222* 787 1956.
18. Segal, H. L.: Kinetic studies on the phenol sulphate conjugating system of rat liver. *J Biol Chem* *213* 161 1955.
19. Tashian, R. E.: Phenylpyruvic acid as a possible precursor of o-hydroxyphenylacetic acid in man. *Science*, *129* 1553 1959.
20. Taussky, H. H.: A procedure increasing the specificity of the Jaffe reaction for the determination of creatine and creatinine in urine and plasma. *Clin Chim Acta*, *1* 210, 1956.

21 WASSMUTH, M.: Über die Oligophrenia phenylpyruvica. Klinische tierexperimentelle und biochemische insbesondere papierchromatographische Untersuchungen. *Ann Univ Saraw* (*Med*), *6* 184, 1958.
22 VESTERMARK, A. and BOSTRÖM, H.: Studies on ester sulphates. 10. On the sulphurylation of mono-, di- and trihydric phenols. *Experientia* *16* 408, 1960
23 WOOLF, L. I.: Excretion of conjugated phenylacetic acid in phenylketonuria. *Biochem J* *49* ix, 1951
24 WOOLF, L. I., GRIFFITHS, R. and MONCRIEFF, A.: Treatment of phenylketonuria with a diet low in phenylalanine. *Brit Med J* *1* 57, 1955.

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Aplastic Anemia Treated with Anabolic Steroids and Corticosteroids[1]

by MARTIN SEIP

Introduction

The treatment of aplastic anemia in childhood has hitherto mostly given discouraging results. Although remissions have occasionally been observed following corticosteroid therapy splenectomy or with supportive therapy only a fatal outcome due to overwhelming infection or hemorrhage has been the rule. Of 40 patients treated at the Children's Hospital in Boston during a 20-year period only one survived after four years. Wolff (4) reported that not more than 3.3 % were cured in a series of 334 patients (including both children and adults) suffering from aplastic anemia

In 1959 Shahidi & Diamond (1 ..) reported promising results with a new therapeutic regimen, using testosterone 1 2 mg/kg body weight per day and small corticosteroid doses (triamcinolone 1.. to 8 mg per day) A beneficial effect was obtained in seven of ten patients, while three died. In discussion following the paper by Shahidi & Diamond, other investigators presented similar experiences in a small number of patients (2).

In 1960 Shahidi & Diamond (3) reported their results in 21 patients, 11 of whom were in remission and an additional three in incipient remission, while five were dead and two unimproved.

We have tried a similar though not identical therapeutic regimen in three cases and have seen a convincing effect in two of them, while one desperately ill patient died a few days after the medication was started. We now use methandrostenolone (Dianabol, Ciba') instead of the high testosterone doses, in order to reduce the incidence of side effects This seems to be as effective as judged by the results in our two cases.

Case Reports

Case 1 A girl, born in 1947 developed signs of aplastic anemia when 7½ years old. Three years later in April 1958, she was admitted to the Children Department A pancytopenia was present with hemoglobin 6 1 g%, .b.c. 2.25 mill., w b.c 2800 with only 500 polymorphonuclears, thrombocytes 28 000, reticulocytes 1.2 2.0 %. The bone marrow was markedly acellular She was treated with corticosteroids and later with splenectomy but only insignificant and transient improvement resulted.

In October 1959 her hemoglobin was between 8 and 9 g%. .b.c. ..5 mill., reticulocytes 1.8 %, w.b.c. 2500 with 350 poly

[1] Based on paper read before Det norske Medicinske Selskab, Jan. 11, 1961

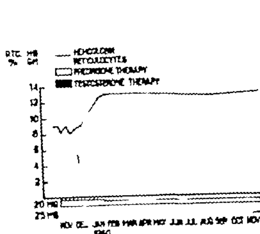


Fig. 1 Hemoglobin and reticulocytes, Case 1

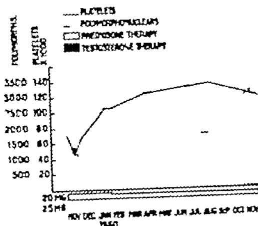


Fig. 2. Polymorphonuclear leucocytes and blood platelets, Case 1

morphonuclears, blood platelets 40,000–50 000

She was started on methyltestosterone ..5 mg and prednisone 20 mg daily on November 3 1959 One month later a reticulocytosis up to 8 5 % developed (Fig 1) Two and a half months after the medication was started her hemoglobin had risen to 1..5 g % b to 3 70 mill w b.c to 5*00 with normal differential count platelets to more than 100 000 The bone marrow was much improved The prednisone dosage was now reduced to 15 mg daily Her blood picture has remained sat sfactory (Figs. 1 and) Her general health is good, and there is no increased bleeding tendency Rather severe acne and hypertrichosis developed on this therapeutic regimen From November *8, 1960 methandrostenolone 10 mg daily has been given instead of methyltestosterone Her blood v lues and whole linical picture is as satisfactory on th form of therapy Her acne is slowly mpro ing

Case A boy born 1934 the third of four siblings. The parent are heal h A sister of the patient died t ears of age from Fanconi anemia following a t o year illness. The immediat ca se of death as profuse bleeding due to thrombocytopenia Treatment with corticosteroids, splenect my and blood transfusions had been ttempted to no avail. The two other siblings are li ing and well

The boy anemia was discovered in March 1960 when he was almost 6 years old One month later he was admitted to the Children Department. His ppearance was remarkably similar to his lat sister s, and he had the same malformations, microcephaly with slight mental retardation (head circumference 49 cm) and pronounced epicanthal folds. His height was at the 10th percentile He was pale and his skin was covered with scattered ecchymoses. A pancytopenia was disclosed with hemoglobin 7 7 g % r b.c 89 mill. w b.c. 3600 blood platelet 23 000 and reticulocytes 1 8 %. Of 3600 leucocytes only 650 were polymorphonuclears Bone marrow aspiration revealed a hypocellular specimen w th depression of ll elements The erythropoiesis wa somewhat better preserved than the myelopoiesis.

The patient was started on prednisone 40 mg daily and methyltestosterone 10 mg daily Three weeks later the prednisone dosage was gradually reduced to 15 mg After 7 weeks on this regimen the condition was almost unchanged, except for moderat reticulocytosis and marked side effect hirsutism, acne flushing of the skin, obesity

On May 31 methyltestosterone was therefore replaced by methandrostenolone

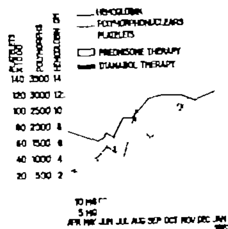


Fig. 3. Hemoglobin, polymorphonuclear leucocytes and blood platelets, Case 2.

(Dianabol), 5 mg daily At the same time the prednisone dosage was reduced to 10 mg daily twelve days later to 7.5 mg. He was then discharged from the hospital and has since then been treated as on out-patient

H was seen again on July 16 and August 10 There was no obvious improvement in the blood picture, but his skin was more normal, his obesity less pronounced. On his next visit t the hospital on September 5 a remarkable improvement had occurred. H looked healthy His hemoglobin had risen to 11.6 g%, b.c. to 3.8 mill., polymorphonuclear leucocytes t 1680 per mm³ (Fig 3) The thrombocytes were still low (15,000)

The same therapeutic regimen has been continued and has resulted in further improvement H is leading a normal lif and has no increased bleeding tendency His hemoglobin level has stabilized around 12 g%. His blood platelets have started t rise and now exceed 100,000 per mm³

Discussion

We have been ble to confirm the observations reported by Shahidi & Diamond that treatment with testosterone and corticosteroids may induce remission in some cases of aplastic anemia in children. In our two cases a definite improvement was noted between two and three months after the therapeutic regimen was started. A reticulocytosis was seen some what earlier more so in one of the patients This is in accordance with Shahidi & Diamond's experience The beneficial effect on the erythropoiesis appeared earlier and was more pronounced than the effect on leucopoiesis and thrombocytopoiesis Eventually significant increases in the number of polymorphonuclear leucocytes and platelets were also obtained. A remarkable increase in bone marrow cellularity occurred. The patients now feel well, have no abnormal bleeding tendency and lead normal lives.

Our observations indicate that the same beneficial effect can be obtained whether methandrostenolone (Dianabol) or testosterone are combined with corticosteroids. The dose of methandrostenolone has been approximately 0.25 mg/kg body weight per day and the corticosteroid doses have been small. The marked side effects which are inevitable when testosterone is given in the large doses recommended by Shahidi & Diamond (1–_ mg/kg body weight daily) can thereby be substantially reduced. Methandrostenolone and similar preparations have the anabolic properties of testosterone while their androgenic effect is slight in the doses used. The risk of premature closure of the epiphyses is probably not important unless the testosterone therapy is continued for years, even so it should be considerably less with methandrostenolone. The observation, if confirmed in a larger series of patients, that the beneficial effect of androgens in some cases of plastic anemia seems to be due to their anabolic rather

than their androgenic properties is of theoretic as well as clinical importance.

It should be stressed that the number of patients treated is still so small that further experience is necessary before a more detailed evaluation of these therapeutic regimens in aplastic anemia is possible Aplastic anemia is not a single disease, and many patients will probably not respond to this new therapy Whether the remissions induced will be permanent is as yet unknown, although Shahidi & Diamond followed their cases up to 3 years.

Whether and when the medication can be safely discontinued is unknown. Shahidi & Diamond discontinued the medication in six patients. One relapsed, while five were in relatively good remissions 4 to 14 months after the treatment was stopped. I would suppose that some patients, e.g those with Fanconi s anemia should be treated over a very long period of time possibly for the rest of their lives, while other patients can be tried without medication after about a year Individualization is certainly necessary A prolonged therapeutic regimen, if indicated, is easier to carry through with methandrostenolone than with testosterone.

It is remarkable that a beneficial effect has been observed in the Fanconi type of aplastic anemia, which has hitherto been invariably fatal.

Summary

Two patients with aplastic anemia (one of them suffering from familial Fanconi anemia) are presented, in whom treatment with anabolic steroids and corticosteroids resulted in marked improvement. Our limited experience indicates that methandrostenolone produces much fewer side effects and may be as effective as testosterone in this condition.

References

1 SHAHIDI, N T and DIAMOND, L. K.: Testosterone-induced remission in aplastic anemia. *Amer J Dis Child*, 98 293–302, 1959

2. — Testosterone-induced remission in aplastic anemia. *Amer J Dis Child*, 98 474–476, 1959

3. — Further observations on testosterone-induced remission in plastic anemia. *Amer J Dis Child* 100 753, 1960.

4 W LFF J A. Anemias caused by infections and toxins, idiopathic aplastic anemia and anemia caused by renal disease. *Pediat Clin N Amer* 4 469–490 1957

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Conditioned Head Rotation Reflexes in Infants in the First Months of Life

by HANUŠ PAPOUŠEK

The experimental approach using conditioned reflexes has produced significant basic data on the early development of higher nervous system activity in infants. Food-seeking reflexes are particularly important in the first months of life, and upon their proper functioning depend health and life during the period of rapid growth in suckling young. For example motor food ingestion reflexes make their appearance by the third intrauterine month in the human fetus (Minkowski 1928) and are fully capable of function at birth.

Among motor food-ingestion reflexes, the conditioned sucking reflex has been thoroughly studied (2, 6, 8 10 19) Upon critical analysis we have observed several basic disadvantages of this method. It is not possible to employ conditioned sucking for estimation of more sensitive indicators of higher nervous activity such as intensity of reaction and latent period furthermore from the methodological aspect, two contradictory trend are apparent with increasing age regressive changes in the sucking reflex occurring at the same time as progressive development of higher nervous activity in infants (13, 14).

For these reasons we have attempted to employ turning of the head toward a source of food as the fundamental reaction for elaboration of conditioned reflexes in infants. In this communication data concerning the use of this new method in study of infantile higher nervous activity will be presented.

Head rotation reflexes are described in the literature as either the seeking component of suckling ("rooting reflex") or as orientational reactions. It is possible to evoke head rotation at birth by tapping the facial nerve at its outlet (3) or the facial muscles (1) Babkin distinguished this reflex movement from the "rooting reflex and orientational head rotation which have their centres in the diencephalon, and did not consider the latter reflexes as inborn. Bechterev & Ščelovanov (2) and Peiper (15) on the other hand, number them among the inborn basic reflexes present at birth. Stirnimann (22) reported that the seeking reflex is less constant than sucking activity in new born infants up to 10-14 days of age, and concluded that this indicated regression in the biological significance of this reflex.

The food-seeking reflex is evoked primarily by gentle tactile stimulation of the



Fig. 1 Position of infant in the recording apparatus.

face in the vicinity of the corners of the mouth The infant usually turns its head quickly toward the stimulating object and attempts to seize it in his mouth. During hunger seeking movements may appear in the absence of any discernable stimulus (17 18) Therefore Popper (16) or Gamper & Untersteiner (7) number them among purposive movements Rooting reflexes may also be called forth by temperature stimuli (5 20 21 23) but not by olfactory stimuli (4 10) According to Peiper (15) natural conditioned reflexes associated with obtaining the bottle develop during the first month of infancy With suitable positioning seeking movements can be evoked in all three axes—vertical, sagittal and transversal After satiation, both conditioned and unconditioned food-seeking activity i suppressed.

Head rotational reflexes in the sense of a conditioned orientational reaction were studied by Kasatkin *et al* (9) who described objective registration of head rotation employing fixation of threads to each temple leading over pullers to pen-writers, one recording movements to the left the other to the right.

Our studies on conditioned food-seeking head rotation were begun in 1956, and an original method was worked out and used throughout these studies (13)

Methods

Recording. The infant lay in a crib fitted with an elastic pad, which allowed registration of overall activity as well as partially unmobilizing the infant so a to eliminat disturbing activity (Fig 1). During movements of the infant the pad oscillated, and

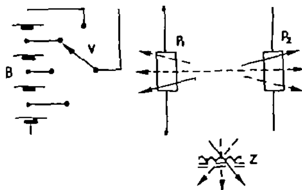


Fig. 2. Diagram of mechanical and electrical registration system (design J. Schemer). B = source of direct current 8 V. P 1, P 2 = potentiometers 50 Ω/2 W. V = potential selector (2–8 V). Z = electromagnetic penwriter 400 Ω.

the oscillation, transmitted through a pneumatic system, was recorded kymographically as an actogram. In order to compare actograms of children of different body weights, special calibration of the kymograph is necessary (Papoušek 1956). Pneumatic registration of respiration was usually carried out simultaneously.

The infant's head was placed in an independent padded cradle which rotated about the horizontal axis with rotation of the head. Two potentiometers, joined as shown in Fig. 2, were placed in this axis with a battery or a rectified transformer as voltage source. Changes in potential occurring with rotation of the head in this axis were registered with a high resistance electric penwriter connected between the slides of the potentiometers. The sensitivity of the recording system was given by the potential applied to the potentiometer circuit (2–8 V). In our system the kymograph penwriter gave deviations of 1 or 2 cm with head rotation of 90° (Fig. 3).

An important factor was the placing of the axis about which the head cradle rotates. A screw fixing the cradle to the axis, could be moved vertically, thereby enabling the cradle to be balanced so that even a newborn infant had no difficulty holding its head in the central, neutral position. On an ordinary crib surface an infant does not hold its head in this position during the first few months of life, but lies with the head turned to one side or the other according to its intrauterine position.

Conduct of experiments. An acoustic stimulus—the sound of an electric bell or buzzer—was used as the conditioning stimulus. This was oriented centrally above the crib. Milk was offered from one side or the other as the unconditioned stimulus. The milk was given by a nurse sitting concealed at the infant's head, and offered in such a way that the infant could not see the nipple without turning its head.

In preliminary observations it was ascertained that the sounds of bell or buzzer were indifferent with respect to this experimental situation. A conditioned reflex to the bell was then elaborated, offering milk from the left. The bell rang for a maximum of 10 seconds before milk was given. If the infant turned its head to the left, it received the nipple immediately and the bell was turned off. If the infant did not seek and receive the milk within 10 seconds, the nurse first touched the left corner of the mouth with the nipple and, if this tactile stimulus was ineffective, turned the infant's head, and placed the nipple in its mouth, after which the bell was turned off.

The experiment was thus arranged so that the more rapidly the infant reacted correctly, the sooner he received the milk. As long as he did not carry out the correct

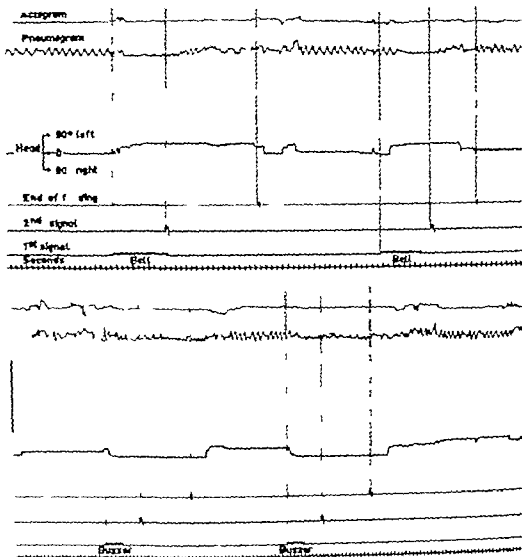


Fig. 3 Example of kymographic record of conditioning of head rotation in 5-month-old infant T.S. From above: actogram, pneumogram, rotation of head, end of feeding, beginning of feeding, conditioned signal, time signal (seconds). The 1st part shows the positive reactions to the bell (to the left), the 2nd part the reactions to the buzzer (to the right).

movement, he received nothing to drink. In this way the practical significance of a correct response was emphasized and probably explains the gradual shortening of the latent period with adaptation to experimental conditions.

When conditioning was so firmly established that the infant carried out 5 successive correct responses, reinforcement of the bell stimulus was stopped, and the extinction of the conditioned reflex studied. Again, when the infant did not react positively to the bell stimulus 5 successive times, the conditioning was renewed, and differentiation of two positive stimuli studied. A new stimulus —a buzzer—was introduced and reinforced

TABLE 1 *Number of stimuli required for elaboration or extinction of conditioned head rotation*

Infant N°	Age at beginning of investigation (days)	Number of stimuli elaboration	b extinction
33	3	435	—
32	17	193	29
31	19	275	24
44	20	73	28
45	20	57	60
43	21	169	35
50	55	28	65
46	56	96	14
41	56	95	46
48	56	73	75
42	56	45	12
49	66	19	—
34	85	69	30
39	85	58	25
37	85	26	37
40	86	85	29
35	88	63	16
36	89	44	17
30	131	41	12
26	140	35	60
47	140	28	34
23	143	40	22
27	146	17	50

by administration of milk from the right side. The criterion of elaboration of differentiation was taken as successful responses to 6 successive stimuli (3 bell and 3 buzzer in random order). After accomplishing this differentiation, the signals were reversed with bell for right side reinforcement and buzzer for the left.

One session was limited to 10 conditioned stimuli as a rule. Care was taken not to have intervals between stimuli the same. A session, once daily, lasted 10–15 minutes. It usually took place at the forenoon feeding time (10–11 o'clock), and before it the infant slept.

In our experience an infant at rest does not move its head more than 30° from the neutral supine position. Rotation of the head 30–60° is taken as a weak positive response and more than 60° as a strong positive response. These angles can be defined with suitably placed contacts or with various types of electrical signals.

Selection of infants. Care was taken that reproducibility of experimental conditions was not interfered with by pathological factors affecting head rotation (postnatal hemorrhage into the sternocleidomastoid, asymmetry of cervical musculature, deformity of the skull, neurological abnormalities etc.). The physiological tendency of the newborn to turn its head on one side, usually the right, did not prove a serious hindrance, since in this apparatus, as already mentioned, even a newborn infant had no difficulty holding its head in the neutral position.

From 1956 to 1958 23 healthy fullterm infants were studied. Throughout the period of study the infants remained under the care of personnel in the research ward for healthy

TABLE 2. *Number of stimuli required for elaboration and double reversal of differentiation between two positive stimuli*

Infant Nº	Age at beginning of investigation (days)	Number of stimuli differentiation	1st reversal	2nd reversal
44	43	31	3	49
43	61	11	44	70
45	6	163	93	49
37	63	149	23	—
4	67	121	23	39
4	8	123	97	73
41	88	56	76	79
48	90	129	47	33
31	97	6	—	—
41	103	105	4	31
39	104	123	59	33
36	105	85	60	18
37	11	85	73	44
35	112	126	—	—
34	119	177	4	49
4	133	51	84	11
9	156	51	—	—
36	163		—	—
79	1 4	47	—	—
47	159	discharged unsuccessful up to the age of 167 days		
46	60			

infant (84.6 days on the average). In all, a total of 13 422 stimuli in 1457 sessions were employed. In one infant elaboration of conditioned reflexes was begun at 3 days of age. The remaining infants fell into 4 narrow age groups at the beginning of the study (Table 1).

Results

Table 1 (a) shows the rapidity of elaboration of conditioned head rotation as measured by the number of stimuli necessary to achieve the given criterion. In infant 33 a stronger stimulus was employed, and experimental sessions were more frequent in order to achieve maximal rapidity of conditioning so that this infant is not comparable to the rest of the group. The first weak positive response was observed on the 5th day of age after 58 stimuli, but not until the 8th day and 83 stimuli did conditioned reflex begin to appear regularly. The criterion of 5 successive positive responses was attained by this infant on the 32nd day of life after 435 conditioning stimuli. In the remaining infants there was a statistically significant direct relationship between age and rapidity of conditioning ($P < 0.01$). Individual differences however appeared in every age group.

The extinction of conditioned reflexes occurred more rapidly than their elaboration, but the rapidity of extinction was related neither to age nor rapidity of original conditioning and individual differences here were not marked. The number of non-reinforced stimuli preceding extinction (by the criterion described above) are also presented in Table I (b).

Table 2 (a) presents data concerning rapidity of differentiation of two positive stimuli in individual infants. The rate was taken as the number of new stimuli (buzzer) preceeding successful differentiation

In almost all children differentiation was learned without difficulty Only infant 46 failed to achieve differentiation even after 50 days. In this infant one had the impression that the learning task was simply too difficult, he reacted more frequently with restlessness during sessions, gave chaotic responses and differentiation remained very weak and erratic. Infant 27 was inadvertently discharged from the ward before achieving this phase of conditioning.

As with primary elaboration of conditioning, a statistically significant dependence upon age was apparent (0 02 > P > 0,01). With increasing age a negative selection of infants began to become of significance since infants who were the slowest to elaborate primary conditioning were thereby oldest at the beginning of differentiation studies We did not, however find any correlation between rapidity of differentiation and rapidity of elaboration or extinction of primary conditioning Individual differences were also apparent in the rapidity of elaborating conditioned reflexes to a new stimulus (buzzer) and in the influence of the new stimulus upon the level of conditioned response to th original stimulus—bell. In addition there appeared interesting individual differences in some phenomena accompanying conditioning (total motor activity respiration, vocal and facial reactions) which require further study

Table (b) shows the rate at which infants achieved differentiation when the two positive signals were reversed—i.e when they signaled reinforcement with milk from the opposite side The rate is expressed as the number of buzzer signals preceeding achievement of the criterion of the differentiation. The number of infants gradually decreased, since it was not possible to keep them all on the ward for a sufficiently long period.

When the infants are arranged by age it is apparent that infants in whom the preliminary phase lasted longer predominate This negative selection was probably the reason why no significant relationship to age was demonstrated in this group. On the contrary there was a tendency for older infants to elaborate this process more slowly With the exception of two infants (44 and 47) the reversal of differentiation occurred more rapidly than the original process of differentiation.

The second reversal of differentiation, in which both signals reverted to their original meaning, was also studied. As can be seen in Table _ (c) the second reversal of differentiation signals was followed by rather faster learning than during the first reversal or original differentiation. Again, no statistically meaningful relation was found to age or to previous phases of the experimental situation.

This method has enabled measurement of latency and intensity of head rotation responses with relative ease The latent period seemed characteristic for individual infants. It tended to decrease gradually with elaboration of the conditioned reflex and with age, but in certain infants was always longer than in others. In the course of differentiation, the latent period first lengthened, particularly in younger in-

TABLE 3 *Individual differences in latency of response*

a = average latency (seconds) of first 10 correct responses
b = " " " of middle 10 " "
c = " " " of last 10 " "

Infant N°	Average latency during elaboration			differentiation			1st reversal		
		b		a	b		a	b	
34	.96	4.65	3.53	2.70	—	2.63	1.50	1.95	0.75
40	2.07	3.5	1.87	1.67	.75	2.43	1.90	1.00	2.00
42	3.33	3.1	1.82	3.40	4.80	—	.96	3.26	3.14
39	2.57	3.17	4.07	.20	1.70	0.66	3.3	.53	1.37
50	4.90	—	5.07	.14	4.80	.48	2.53	2.83	2.50
47	5.33	—	4.96	.76	3.03	3.02	3.4	3.16	3.31
45	5.44	6.43	6.22	2.36	5.36	2.96	2.18	2.22	1.65
44	5.80	3.91	4.96	4.36	4.41	3.36	3.59	3.41	3.57

fants, and then again decreased. In addition, the latent period was found to reflect temporary influences sensitively—the degree of hunger various changes in the dynamic stereotype and experimental conditions, the course of the previous response etc

A detailed analysis of these associated subtle indicators of the functional state of higher nervous activity deserves a separate communication Here for illustration only changes in individual latent periods and their changes in the course of investigation are sh wn in Table 3

In general the remarks above apply to the intensity of response In our experience however intensity of reaction is a less sensitive indicator than lat nt period In this case, we also observed dependence upon age, the degree of elaboration of conditioning and individual characteristics. In younger infants unclear reactions appeared first, followed by weak responses which gradually strengthened In older infants reactions were sometimes very striking, even after only few reinforcements. During extinction of conditioned reflexes the intensity of response gradually decreased Intensity often decreased temporarily during differentiation and reversed differentiation

In addition to intensity and latency of reactions, it was also possible to estimate the rapidity with which the infant turned his head. It has been a frequent observation that a strong rapid reaction had a short latent period and conversely

Discussion

These studies have confirmed that reflex rotation of the head toward a source of food is advantageous methodologically for the investigation of conditioned food-seeking reflexes even in newborn infants. In our results, the influence of age upon elaboration of conditioned reflexes has been found to be quite consistent, unlike results using the sucking reflex which simplifies analysis of developmental processes in higher nervous activity

It has been found that data on basic indices of higher nervous functions can be obtained, even in the first half year of life. A sufficiently well-established conditioned reflex can be elaborated by the *beginning of the 2nd month*; differentiation between two positive stimuli by the 3rd month and reversal of differentiation by the 4th month. The latter was even achieved, in one infant, in the 3rd month of life. In addition to data on the rapidity of the conditioning process, this method has also permitted study of more subtle indicators of cortical function, such as the latency and intensity of conditioned responses. This experimental approach has the added advantage that the infant receives milk to drink only when it carries out the correct motor reactions, which is a powerful impulse toward shortening of the latent period of response with adaptation to the experimental situation. We believe this experimental approach to have more than purely methodological interest, for it has already yielded interesting data on latency of motor responses at this age which as yet have not been described. Observations on the relatively complex differentiation of which 3 or 4 month old infants are capable have not as yet been reported.

The circumstance of elaboration of two analogous conditioned reflexes—rotation of the head to the left or right—opens important new methodological possibilities. First one can study elaboration of two reinforced stimuli. This is superior to using differentiation between a reinforced and non-reinforced stimulus in that it does not evoke the marked restlessness often produced by, for example, frustration of the sucking reflex when milk is not forth coming. It also permits comparison of two symmetrical reflexes under ideally comparable conditions, observation of the influence of the mode of reinforcement, etc.

This method also opens the way to a study not of pure motor responses alone but of the development of purposeful adaptive activity as a whole. Using the *experimental situation described*, reflex rotation of the head may be given the meaning of a purposeful food seeking response, an orientational reaction, or even a defense reaction. One can study how a simple basic movement, easily registered, develops into a complex adaptational response to which an infant imparts various meanings with various stimuli. To our knowledge analysis of purposeful activity at such an early age does not appear in the literature. For that matter it has not been suggested that 3–4 month old infants are even capable of such complex reactions as appeared in these observations. This is probably because heretofore coordination of activity in limbs, particularly the upper, has usually been studied. However, if one studies head movements which according to the principle of cephalocaudal development of motor activity mature sooner than movements of extremities, differentiation of motor reactions can be detected earlier than has been hitherto supposed.

The results of the investigation using conditioned head rotation may be summarized as follows:

1) Conditioned rotation of the head toward a source of food may be used for the study of conditioned reflexes in infants the first few months of life. Responses are of [illegible] and easily registered

kymographically Analysis of records is simple Registration of head rotation and total motor activity may be carried out simultaneously

2) It is possible to elaborate a sufficiently stable conditioned reflex, on the basis of unconditioned head turning by 5–6 weeks of age Weak and unstable conditioned responses were observed in one infant in the 2nd week of age.

3) It i possible to elaborate differentiation between two positive stimuli The infant thus elaborates two symmetrical hetero-lateral but entirely analogous reflexes. One stimulus is reinforced by giving milk from the left the other from the right Such differentiation was acheived in one infant the 2nd month but in the majority it appears during the 3rd month. By the 6th month it was possible to finish two reversals of differentiation of two positive stimuli. In one infant this was accomplished by the end of the 2nd month

4) The original elaboration of a conditioned reflex and differentiation of two positive stimuli is dependent upon age, and this age correlation is statistically significant—the older the infant, the more rapid conditioning or differentiation. Extinction of conditioning and renewal of the conditioned response on the other hand, are not related to age No relationship with age was apparent in reversal of differentiation since here a negative selection of individual infants operated (those infants slowest at achieving the preliminary stages of conditioning reached this phase at the oldest ages)

5) In addition to data on rapidity of conditioning, this method has also yielded more subtle indications of higher nervous activity—latency and intensity of conditioned responses. Observations of the latency of motor reactions in very young infants were obtained. Latency decreases as conditioning progresses more rapidly in older infants. Changes in latency were considered to be manifestations of cortical adaptation to the experimental situation, and the situation was designed to demonstrate this, since the rapidity with which the infant carried out the correct response determined the rapidity with which it obtained milk to drink.

6) Employment of conditioned head rotation, as described here, is suitable for other than the study of conditioned food seeking reflexes. Since it is possible to elaborate two entirely analogous symmetrical reflexes under ideally comparable conditions, it permits comparison of the influence of modification of the conditioning stimulus unconditioned reinforcement and other experimental conditions

This experimental approach is also promising for the study of purposive activity Head rotation is singularly suited to the study of how a basic responsive movement becomes modified under various conditions Head rotation may function in various circumstances as a component of food-seeking activity orientation or defense reactions A further advantage lies in the fact that purposive head movements by the principle of cephalocaudal development, appear earlier than those of extremities and are also easier to register

Summary

A new experimental system for study of higher nervous function has been tested with 23 normal fullterm infants. This method employs conditioned rotation of

the head recorded electro-mechanically. As compared with conditioned sucking studies, this method has been found advantageous from several points of view.

New data on the development and coordination of motor responses in young infants have been obtained. A sufficiently stable conditioned reflex may be achieved at 4–6 weeks of age; differentiation of two positive stimuli at 3 months and double reversal of this differentiation in the 4th month. The experimental approach described permits study and comparison of two analogous symmetrical heterolateral reflexes and simple analysis of intensity and latency of response as sensitive indicators of cortical functions. It also opens new possibilities for the study of early purposive motor reactions. Since rotation of the head may take on various meaning —food-seeking, orientation, defense—it is particularly well suited to the study of ontogenetic change of one simple motor act into a complex adaptative mechanism.

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References

1. Babkin, P. S.: Ščečno-vraščatel'nyj refleks golovy u grudnych detej (Facial rooting reflex in infants). *Ž Nevropat Psychiat Im Korsakova*, 3 662 1953.
2. Bechterev, V. M. and Ščelovanov, N. M.: K obosnovaniju genetičeskoj refleksologii (The motivation of genetic reflexology). *Novoje v refleksologii*, 1 116 1925.
3. Catel, W.: Normale und pathologische Physiologie der Bewegungsvorgänge im gesamten Verdauungskanal. G. Thieme, Leipzig 1926.
4. Canestrini, S.: Über das Sinnesleben des Neugeborenen. Springer, Berlin 1913.
5. Darwin, Ch.: Biographische Skizze eines kleinen Kindes. Kleinere Schriften, 2. E. Krause, Leipzig 1886.
6. Denisova, M. P. and Figurin, N. L.: K voprosu o pervych sočetatel'nych piščevych refleksach u grudnych detej (The first conditioned food reflexes in infants). *Vopr Genet Refleksol Pedolog* 1 61 1929.
7. Gamper, E. and Untersteiner, F.: Über eine komplex gebaut postencephalitische Hyperkinese und ihre möglichen Beziehungen zum oralen Einstellungsmechanismus. *Arch Psychiat*, 71 282, 1924.
8. Kasatkin, N. I.: Rannije uslovnyje refleksy v ontogeneze u čeloveka (Early conditioned reflexes in human ontogenesis). Medgiz, Moscow 1948.
9. Kasatkin, N. I., Mirzojanc, N. S. and Chochitva, A. P.: Ob orientirovočnych uslovnych refleksach u detej pervogo goda žizni (The conditioned orientation reflexes in infants during the first year of life). *Ž Vys Nerv Dejat*, 3 192, 1953.
10. Marquis, D. P.: Can conditioned responses be established in the newborn infant? *Ped Sem & J Genet Psychol* 39 479 1931.
11. Minkowski, M.: Neurobiologische Studien an menschlichen Früchten. *Abderhaldens Hb Biol Arbeitsmeth* V B 511 1928.
12. Papoušek, H.: Aktografie malých kojenců (Activity recording in small infants). *Čs Pediatrie* 11 550, 1956.
13. — Metodika issledovanija piščevych uslovnych refleksov u detej v vozraste do šesti mesjacev (Method of studying conditioned food reflexes in infants during the first six months of their life). *Ž Vys Nerv Dejat* 9 143, 1959a.
14. — Podmíněné motorické potravové reflexy u kojenců (Conditioned food-seeking reflexes in infants). Dissertation. Charles University Prague 1959b.
15. Peiper, A.: Unbedingte und bedingte Reflexe der Nahrungsaufnahme. *Kinderärztl Prax*, 11 507 1958.
16. Pötzl, O.: Studien über Saugphänomene. *Arch Psychiat Nervenkr*, 1 221 1921.
17. Prechtl, H. F. R.: *Die Kletterbewegungen*

beim Säugling. *Mschr Kinderheilk* *101* 519 1953
18. Preyer, F Die Seele des Kindes. Th. Grieben, Leipzig 1895 4th Ed.
19 Ripin R. und Hetzer, H.: Frühestes Lernen des Säuglings in der Ernährungssituation. *Z Psychol* *1* 2 ..., 1930
20. Schwendel, F Wärme- und Kältereaktionen von neugeborenen Säuglingen. Dissertation. Greifswald, 1945
21 Sikorski, I A.: Die seelische Entwicklung des Kindes. Leipzig 1908.
22. Stirnimann F Die Einstellreaktion beim Neugeborenen. *Jb Kinderheilk*, *1/6* 224, 1937
23. — Das Kind und seine früheste Umwelt. S Karger Basel 194

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Studies of the Immunological Relationship between the Antigens from Different Organs in Human Ulcerative Colitis[1]

by OVE BROBERGER

In a previous publication [1] it was reported that sera from children with ulcerative colitis usually contain antibodies which react with extracts from human colon, liver or kidney The antibodies could be demonstrated by means of gel diffusion and haemagglutination techniques and behaved electrophoretically as gamma-globulins. The tissue extracts were prepared by extraction with phenol-water at 65 C and were supposed to contain lipopolysaccharides. This assumption was further substantiated by the fact that the extracted tissue components could be adsorbed onto sheep red cells without previous treatment of the cells with tannic acid [.].

Since the sera reacted with extracts from both colon, liver and kidney it was desirable to learn whether the antigens from the different tissues were immunologically identical, related (cross-reacting) or different Previous experiments by means of gel diffusion techniques had given certain indications that they could be identical or cross-reacting [1]. However gel diffusion cannot be regarded as a sensitive tool for the study of antigenic identities in cases where knowledge of the optimal concentrations of antigen and antibody is not precise This communication gives the results of a study of such relationships by means of haemagglutination inhibition.

Methods

Source and preparation of antigen Colon liver and kidney were obtained within one hour after death from children (blood group O) who had died during their first day of lif The organs were extracted with phenol-water at 65°C as described elsewhere [1]. For coating of the red cells the lyophilyzed extracts were dissolved in buffered saline (phosphat buffer pH 4) in a concentration of 0.5 mg/ml.

Antiserum Serum from a child with ulcerati colitis was used as antiserum. It reacted with colon extract in a dilution of 1 64 and with liver extract in a dilution of 1 32. Before use the serum wa inactivated at 56°C for 30 minutes in order to destroy complement Heterophile antibodies were removed by repeated absorption with washed sheep erythrocytes.

Haemagglutination nhibition. Sheep erythrocytes were coated with colon or liver extracts described lsewhere [1]. The titrations were performed on perspex trays (*MRC* pattern) Serial dilutions of serum were made in buffered saline and aliquots of 0 1 ml of the dilutions were placed in the rows of holes on the tray One-tenth of a millilitre aliquots of the antigenic extract wer then added using a different concentration of antigen for each row of serum dilutions

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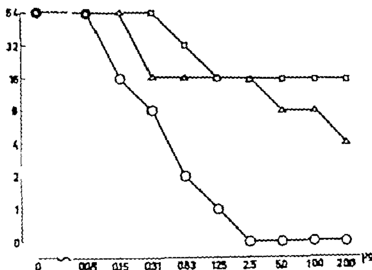


Fig. 1 Haemagglutination inhibition of sheep erythrocytes coated with colon extract after absorption of antiserum with colon liver and kidney extract *Abscissa* Amount of antigenic extract (μg) used for absorption of 0.1 ml of antiserum. *Ordinate* Serum titre given as the reciprocal of highest serum dilution giving macroscopically visible haemagglutination *Circles* Haemagglutination of colon coated cells after absorption of antiserum with colon extract *Triangles* After absorption with liver extract *Squares* After absorption with kidney extract.

Antigen dilutions were made in twofold steps from 1:1 (0.2 mg/ml) to 1:256. In controls, 0.1 ml of buffered saline instead of antigen was added to each of the serum dilutions. For absorption the mixtures were incubated at 37 C for 60 minutes. One-tenth of a millilitre aliquots of a 0.25 % suspension of antigen-coated sheep erythrocytes (standardized in a Beckman spectrophotometer after haemolysis) were finally added to all samples. The trays were shaken and kept at 37 C for 60 minutes. After a second shaking the trays were left at 4°C overnight. The settling pattern of the red cells gave some indication of the degree of haemagglutination. However the final judgement was always made on the appearance of the red cells after a gentle shaking. The serum titer is given as the reciprocal of the highest serum dilution giving macroscopically visible haemagglutination.

Results

As can be seen from Fig. 1 unabsorbed serum caused haemagglutination of sheep erythrocytes coated with *colon* extract in a serum titer of 64. When the serum was absorbed with 2.5 μg of colon extract or more the haemagglutination was completely inhibited. Using liver extract for absorption, complete inhibition could not be obtained under the present experimental conditions. The curve represented by squares reveals that the inhibitory capacity of the kidney extract was even less than that of the liver extract.

Fig. 2 shows the results of another experiment when the sheep red cells were previously coated with *liver* extract. In this case the titer of the unabsorbed serum was 32. As is apparent from the shape of the curves, no significant difference between the inhibitory capacities of the colon and liver extract could be noted in an experiment of this type. Unfortunately the small amount of kidney extract available prevented additional experiments with kidney-absorbed serum or with kidney-coated cells.

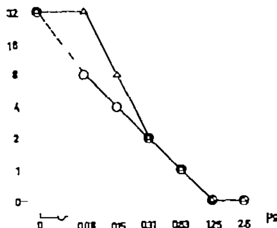


Fig. — Haemagglutination inhibition of sheep erythrocytes coated with liver extract after absorption of antiserum with colon and liver extract. *Abscissa*. Amount of antigenic extract (μg) used for absorption of 0.1 ml of antiserum. *Ordinate*. Serum titre given as the reciprocal of highest serum dilution giving macroscopically visible haemagglutination. *Circles*. Haemagglutination of liver-coated cells after absorption of antiserum with colon extract. *Triangles*. After absorption with liver extract

Discussion

There are several explanations available for the results of the haemagglutination experiments depicted in Fig 1 Thus one could assume that haemagglutination is brought about by the reaction of the antibodies with an immunologically identical antigen occurring in both colon, liver and kidney extracts. The greater inhibitory capacity based on the amount of lyophilyzed powder used, of the colon extract as compared with that of the liver or kidney extracts would then be due to a larger amount of antigen in the colonic extracts. Secondly the antigens occurring in the three organs could be different but immunologically related. In this case, haemagglutination inhibition would have been caused by the presence, in the liver and the kidney extracts of antigens which cross-react with the antibodies against the colon antigen. With other word the results obtained in Fig 1 with liver or kidney extracts, could be explained on the basis of a lesser fit of these antigens with th anti-colon antibodies. However a third possibility has also to be taken into consideration. In addition to antibodies against one or several common antigens present in colon, liver and kidney the sera may also contain antibodies against antigen(s) present only in the colon. This would fit with the incomplete inhibition obtained with the liver and kidney extracts in the experiments of Fig. 1 Moreover th s explanation is favoured by the results given in Fig. 2 The very similar inhibition curves obtained here with the colon and liver extract seem to indicate that only a fraction of the antibodies which participate in the haemagglutination of colon-coated cells are involved in the haemagglutination of *liver-coated* lls. In any case the complete inhibition of haemaggl tination of *liver-coated cells* after absorption with *colon extract* also suggests that these two organs have at least one immunologically identical or similar antigen in common

The occurrence of antibodies in the sera of patients with ulcerative colitis, against several unrelated antigens would account for the fact noted previously [1] that not all sera which react with colon extract also react with liver or kidney extract However the insufficient amount *of antigen available has so far prevented* an exploration of this problem Any definite conclusions as to a possible multiplicity of the antigens and the antibodies have to await the results of further experiments.

Summary

Sera from children with ulcerative colitis haemagglutinate sheep red blood cells coated with phenol–water extracts of human colon, liver or kidney It was found that the haemagglutination of colon-coated cells could be completely or partially inhibited by previous absorption of the sera with extracts of colon, liver or kidney Likewise the haemagglutination of *liver coated cells could be inhibited by means* of extract from colon or liver The results indicate that liver and colon contain at least one immunologically identical or similar antigen. In addition, certain preliminary results suggest that the sera contain antibodies against several distinct antigens some of which are present only in the extracts of colon and not in those of liver or kidney

References

1 Broberger, O and Perlmann P.: Autoantibodies in human ulcerative colitis. *J Exp Med 110* 61 1959

2 Rhodes J. M. Sorkin E. and Boyden S. V Studies on haemosensitin from Mycobacterium Tuberculosis in zone electrophoresis. *Biochim Biophys Acta, 2* 403, 1957

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Concentration of Fibrinogen in the Plasma of Healthy and of Erythroblastotic and Hyperbilirubinemic Newborn Infants

by LARS ENGSTRÖM and LARS KAGER

Taylor (5) presented the data from the literature on the concentration of fibrinogen in the plasma of newborn infants. The mean values reported in the various studies range from 140 to 350 mg/100 ml with individual observations from 60 to 800 mg/100 ml The discrepancies are thus considerable Taylor also made observa tions of the concentration of fibrinogen in the plasma during the first week of lif of 45 healthy infants. H used the clot density technique of Losner *et al.* ( ) and found a slight increase in mean concentration of fibrinogen during the first three days of life (First day mean 231 and range 182–393 third day mean 264 and range 196–300) Neither sex, birth weight, nor concentration of fibrinogen in the mother s plasma had any influence on the infant s initial concentration of fibrinogen. Taylor discussed the possibility of fibrinogen con centration being used as a measure of an infant s liver function.

Rice (4) reported an immature infant (birth weight 1900 g) with erythroblastosis and petechiae on the abdomen and face and with blood tinged sputum. The clot ting time was two hours. Addition of thrombin in excess resulted in the forma tion of a fine fibrinous network. After exchange transfusion of ₋5–35 % of the blood, the plasma fibrinogen was 120 mg/ 100 ml Rice concluded that the quantity of fibrinogen *before* the transfusion had either been appreciably lower or that the blood had been initially free of fibrinogen.

Proceeding from Taylor s and Rice s results we have investigated whether there is any difference between the concentra tions of fibrinogen in the plasma of healthy newborn infants and in infants with erythroblastosis or hyperbilirubinemia without blood-group incompatibility

Material and Methods

Samples for f brinogen study were drawn from 39 infants by means f a polyethylene catheter introduced into the umbilical vein. Twenty of the subjects were healthy full-term infants; the samples from fourteen of these infants were collected within two hours after birth, while in the other six the study extended over the first three to six days of life.

Ten infants had erythroblastosis due to Rh or ABO immunization of the mother In these cases the samples were collected within two hours of birth and *before* an exchange transfusion.

TABLE 1 *Fibrinogen concentration in plasma of erythroblastotic infants at birth (mg/100 ml)*

Degree of severit	N of cases	Fibrinogen
Group 1		
Coombs(+) 0 exchange transfusion	1	270
Group 2		
1 xchange transfusion	4	190, 290 40, 310
Group 3		
ex hange transfusions	2	240 300
Group 4		
3 or more xchange transfusions	3	*83, 290 250

Groups 1–4

No. of cases	Range	Mean	SE	SD
10	190–330	271	14 7	46.3

Nine infant had exchange transfusions performed because of hyperbilirubinemia without blood-group incompat bility Sam ples for f brinogen determination were drawn before the first transfusion which was between the third and sixth day f lif

The f brinogen was determined by Mor rison s syneresis method (3) as modified by Blomback (1) a method not previously used in determination of f brinogen in the plasma of newborn infants. The anticoagulant used in the collection f the samples consisted of 3.8 trisodium trat (H O) in a dilution of one part (nine part f blood. To every ml of trisodium citrat 40 mg of lysin ethyl ester dihydrochloride were added so that any f brinolytic tivity present in the sample was eliminated (1 0)

In 33 infants hematocrit alues were determined concurrently with collection of the sample for f brinogen determination (centrifugation for 5 min tes t 10 000 p.m.)

Results

A. *Healthy full-term nfants xam ned within two hours of birth*

Individual observations ranged from 175 to 285 mg/100 ml, giving a mean value of 225 (Standard error ±10 0 standard deviation ±37.3 number of cases 14.) No difference was found between boys and girls.

One infant who was excluded from the normal material because of mongolism had the lowest fibrinogen concentration in plasma of any infant studied, i.e. 1*0 mg/100 ml

B *Healthy full-term infants examined during third to sixth day of life*

The mean was 322 lowest *40 and high est 380 mg/100 ml. (Standard error 1– standard deviation 51.9 number of cases 6.)

C *Erythroblastotic infants*

These infants are classified in four groups in Table I according to the severity of the condition. Group 1 comprises the mildest cases whose Coombs test was directly positive but who did not require an exchange transfusion. The infants in Group ~ received one exchange transfu

TABLE _. *Fibrinogen concentration in plasma of infants with hyperbilirubinemia without blood-group incompatibility*

Case no.	Non-conjugated bilirubin, mg/100 ml	Fibrinogen mg/100 ml
1	24.0	193
	17.1	200
3	1.9	223
4	20.2	270
5	20.8	295
6	4.3	290
7	23.1	3[illegible]0
8	19.5	415
9	21.3	445

Case 1 9

N of cases	Range	Mean	SE	SD
9	193–445	297	30.0	89.8

tion, in Group 3 two, and in Group 4 at least three. The most seriously affected children are thus those in Groups 3 and 4. The table also shows the fibrinogen concentration at the time of the first transfusion. The individual values ranged from 190 to 350 mg/100 ml with mean 271 (Standard error 14.7, standard deviation 46.3, number of cases 10.)

D. *Hyperbilirubinemic infants without blood-group incompatibility*

The severity in terms of the non-conjugated serum bilirubin concentration at the time of the first exchange transfusion, which immediately followed the drawing of the sample for fibrinogen study, is shown in Table 2. The fibrinogen concentrations ranged from 193 to 445 mg/100 ml, mean 297. (Standard error 30.0, standard deviation 89.8, number of cases 9.)

In 33 cases the plasma fibrinogen concentration was correlated to the hematocrit value at the time of drawing the sample without regard to the above classification (Table 3).

Discussion

The results show that the concentration of fibrinogen in plasma was not lower in erythroblastotic than in healthy full-term infants. Indeed the most severely affected infants proved to have fairly high fibrinogen levels.

Statistical analysis revealed that there was no correlation between hematocrit value and fibrinogen concentration as shown in Table 3. Thus a low hematocrit value in conjunction with erythroblastosis did not affect the fibrinogen concentration.

Samples for fibrinogen determination were drawn within two hours of birth. No account has been taken in this study of the possibility that the fibrinogenolytic activity in erythroblastic infants might become elevated after birth. Hemorrhagic tendencies were not observed clinically in any case.

Proceeding from Taylor's postulate that the plasma fibrinogen may be an index of the liver function at birth, we determined the concentration of fibrinogen in

TABLE 3 *Relationship between haematocrit value and plasma fibrinogen concentration*

Case no.	Hematocrit	Fibrinogen mg/100 ml	Case no	Hematocrit %	Fibrinogen, mg/100 ml
1	32	300	18	53	193
[illegible]	35	330	19	56	283
3	33	310	20	56	340
4	35	290	21	56	323
5	42	283	22	57	[illegible]00
6	42	380	23	57	270
7	46	340	[illegible]4	57	370
8	46	443	[illegible]5	58	190
9	46	290	26	58	290
10	47	40	[illegible]7	59	415
11	49	210	28	58	230
1[illegible]	50	3[illegible]5	29	60	175
13	50	350	30	60	370
14	32	230	31	61	190
15	34	215	3[illegible]	62	[illegible]75
16	33	230	33	70	170
1[illegible]	63	40			

the plasma of a group of infants with neonatal hyperbilirubinemia without blood group incompatibility The indication for exchange transfusion was here an elevated, non-conjugated bilirubin level in serum (Table) i e an expression of inadequate hepatic capacity Since the transfusions and determinations of fibrinogen concentration were not done in these infants until the third to sixth day of life the fibrinogen concentration was also studied in a group of infants of the same age but without icterus No change was observed in plasma fibrinogen as a result of elevated non-conjugated serum bilirubin Nor was there any correlation between fibrinogen concentration and the quantity of non-conjugated bilirubin.

Apart from the fibrinogen value of the mongoloid infant all fibrinogen levels fell within the limits found by Taylor for healthy children during the first week of life

Our results, therefore do not support Rice's hypothesis that Rh immunization is accompanied by a low concentration of fibrinogen in the plasma of the affected infant—nor that the fibrinogen concentration changes as a result of hyperbilirubinemia not caused by blood group incompatibility This does not exclude the possibility of course that severe cases of erythroblastosis or hyperbilirubinemia may be complicated by afibrinogenemia due for example to intravasal clotting or fibrinogenolysis without direct association with the basic disease In erythroblastosis and hyperbilirubinemia in general, however the fibrinogen concentration in plasma is not a test of functional value though this does not preclude the necessity of complete investigation of coagulation including determination of fibrinogen concentration in the plasma in all cases of clinically observed hemorrhagic diathesis in infants. But, as already stated, we had no case of hemorrhagic diathesis in our material.

Summary

The concentration of fibrinogen in the plasma of 39 infants during the neonatal period was determined by Morrison's syneresis method as modified by Blombäck. The samples were drawn from the umbilical vein by catheterization. The results, expressed in mean and standard error of the mean, were as follows.

A. Healthy full term infants at birth. 225 ± 10.0 mg/100 ml.
B. Healthy full term infants during third to sixth day of life: 322 ± 11.2 mg/100 ml.
C. Erythroblastotic infants at birth. 271 ± 14.7 mg/100 ml.
D. Hyperbilirubinemic infants without blood-group incompatibility during third to sixth day of life: 207 ± 30.0 mg/100 ml.

No statistically significant difference was found between healthy and erythroblastotic or hyperbilirubinemic infants as regards concentration of fibrinogen in the plasma.

References

1. Blombäck, B.: On the properties of fibrinogen and fibrin. *Ark Kemi, 12* 99 1958.
2. Lorber, S., Volk, B. W., Jacobi, M., and Newhouse, S.: Spectrophotometric studies on clot density. *J Lab Clin Med 38* 23, 1951.
3. Morrison, P. R.: Preparation and properties of serum and plasma proteins IV. Some factors influencing the quantitative determination of fibrinogen. *J Amer Chem Soc 69* 2723 1947.
4. Rice, W. G.: Deficient prothrombin and fibrinogen in fetal erythroblastosis fetalis. *J Pediat, 42* 231 1953.
5. Taylor, P. M.: Concentration of fibrinogen in the plasma in normal newborn infants. *Pediatrics, 19* 233, 1957.
6. Troll, W., Sherry, S., and Wachman, J.: The action of plasmin on synthetic substrates. *J Biol Chem 208* 85, 1954.

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Arteriographic Studies in Children with Cerebral Palsy

by S. BRANDT S. BRÜNNER and V. WESTERGAARD-NIELSEN

Cerebral angiography is being used to an increasing extent in neurologic disease because it affords such an excellent supplement to other diagnostic methods. Until the percutaneous method of injecting the contrast material was described about 15 years ago there was marked reluctance to subject children to angiography. For this reason pneumoencephalography has been preferred where information on cerebral pathology was wanted in children. Recently ta papers on encephalographic f d n in spastic children have been p hl led (3 9). On the other hand, a g graphic studies on children with cerebral palsy or studies correlating angiographic with encephalographic findings have not been reported although some recent authors have reported in general on carotid angiography in children (6 7 10 11). We have therefore collected a series of 24 patient from the Cerebral Palsy Clinic at the Orthopaedic Hospital in Copenhagen in whom arteriographic and encephalographi studies had been performed in some of the radiological and neurosurgical departments in Copenhagen and at the Radiological Department of the Copenhagen County Hospital in Gentofte usually prior to the time of examination at the Cerebral Palsy Clinic.

Because of the widely different pathology it was anticipated that the angiograms would not show specific changes; indeed the findings were protean. Nevertheless we felt that it might be of interest to compare the angiograms with encephalograms obtained at approximately the same time.

Material

The angiograms were mainly obtained from children with cerebral palsy of post natal origin because patients of this cat gory re more likely to be examined in neurosurgery departments where angiography is a rather routine diagnostic procedure. In series of 628 children with cerebral palsy examined at the Cerebral Palsy Clinics at the Orthopaedic Hospitals in Copenhagen and Aarhus, Brandt & Westergaard Nielsen (1) found 13% postnatal cases, whereas in the present series 14 of 24 children i.e. little over one-half were postnatal. Six were congenital (i.e. presumably prenatal) and only four perinatal in onset. The distribution f patients on the basis of the nature and extent of the disturbance of coordination is shown in Table 1. The series comprises eight girls and 16 boys who ranged in ge from 8 months to 15 years at the time of the angiographic examination. Only four were under 3 years of age.

TABLE 1 *Symptomatic classification*

Spastic (= pyramidal)			19
Left sided	9		
Right-sided	5		
Predominantly left-sided	4		
Predominantly right-sided	0		
No difference between right and left	1		
Athetotic (= extrapyramidal)		2	
Mixed type		3	
Total		4	

TABLE * *Arteriographic findings in patients with ventricular dilatation*

Elevation, distension, nd possibly shift of the anterior cerebral artery	8
Vascular malformation	
Thrombosis in the anterior cerebral artery	1
Agenesis of the corpus callosum	
Normal	3

Technique

The 24 patients underwent a total f 40 carotid angiographies, 29 right-sided and 11 left-sided. All the examinations were performed by the percutaneous technique under general anaesthesia.

Encephalography had been performed by the suboccipital or lumbar rout or as ventriculography a total f 30 times. Two patients did not have encephalography As is pparent from the figures, these procedures were repeated in a majority f the patients. Eight had two angiographies, five had three, and two had four angiographic examinations.

Results

(a) *Patients with ventricular dilatation*

In 18 out of the 4 children, encephalography revealed enlargement of the ventricular system. It was symmetrical in seven whereas in seven there was right sided and in four left-sided dilat tion only These 18 children had a total of 5 right sided and five left-sided angiographic examinations. The uneven distribution of right and left-sided procedures is in accordance with the fact that in children with cerebral palsy there is a preponder ance of postnatal, left-sided. spast c hemiplegia (1). The arteriographic findings in these patients are summarized in Table .

From the table it may be seen that of 18 patients with ventricular dilatation, pathologic arteriographic findings were found in 13 a relatively high proportion possibly because of the somewhat stricter indications for arteriography in children.

In one of the cases in the first group (elevation, distension and possibly shift of the anterior cerebral artery) it had been suggested in the report of the ar arteriograms—supported by X ray skeletal changes—that there might be an expansive lesion. This was later proved. Another case in the same group came to autopsy 15 months after the last radiological examination, an angioma racemosum arterio-venosum was found in the right temporal lobe and a haematoma in the corpus striatum on the right with bleeding into the right lateral ventricle Furthermore there was hydrocephalus (the case has been reported by Christensen & Brandt ( )). Even with the knowledge of the post mortem findings, the lesion could not be demonstrated in a review of the right-sided angiograms however the afferent vessels especially the middle cerebral artery were rather prominent for a 1 year-old baby and the efferent vessels were extremely well developed. This finding demonstrates how difficult it may be to visualize angioma racemosum on the angiograms obtained

by the usual three film technique owing to the great speed at which the blood runs through the numerous arterio-venous shunts (5, 8). The chances of catching an angioma racemosum are considerably greater when using rapid serial angiography (4).

(b) *Patients without ventricular dilatation*

Four out of the 4 children showed no signs of ventricular enlargement or other encephalographic abnormalities. In three of these cases the angiograms were normal while one showed periarterial flow of contrast with non filling of the anterior cerebral artery.

(c) *Non-encephalographed patients*

As already mentioned two children did not have encephalography. These two angiographies also showed the combination of periarterial flow of contrast with non filling of the anterior cerebral artery.

(d) *Patients with clinical hemiplegia*

All the children with *left-sided* spastic hemiplegia belonged to the group with bilateral or right-sided dilatation of the ventricular system. In addition one child of this category did not have encephalography. The ateriographic findings in this group of left-sided spastic hemiplegia are summarized in Table 3.

Two characteristic cases illustrate the findings in this group.

The first patient was a boy aged 1 months, who had been delivered by caesarean section. From the age of 2 months he began to have clonic twitching in the left arm, and spastic left-sided hemiplegia was noted. His general development was retarded. At

TABLE 3. *Arteriographic findings in patients with clinical left-sided hemiplegia*

Distension and elevation of the right-sided anterior cerebral artery	1
Aneurysm of the right internal carotid	1
Shift of the right anterior cerebral artery to the right	
Thrombosis of the right internal carotid	
Normal	3

the age of 4 months he had subdural puncture evacuating a hygroma. Ventriculography (Fig. 1) showed ample surface air on the left, no surface air on the right, a slight shift of the ventricular system to the right and dilatation mainly on the right. Angiography at the age of 21 months revealed displacement of the anterior cerebral artery to the right. Both anterior cerebral arteries filled. By compression filling was also obtained of the left middle cerebral artery. The peripheral vessels in the area supplied by the right medial cerebral artery did not extend quite to the tabula interna. In view of the displacement to the right of the anterior cerebral artery these findings suggest cortical atrophy and rather exclude a recurrence of the hygroma.

An 11 year-old boy had been in good health until the age of 9 when he sustained two cranial injuries in rapid succession. He then developed left-sided spastic hemiparesis. Right-sided carotid angiography revealed saccular aneurysm at the bifurcation of the internal carotid artery. Craniotomy was performed, excising the aneurysm and clamping the anterior cerebral artery with clips (Fig. 2).

TABLE 4. *Arteriographic findings in patients with clinical right-sided hemiplegia*

Suspicion of astrocytoma in the left temporal lobe	1
Shift of the anterior cerebral artery to the contralateral side	1
Normal	3

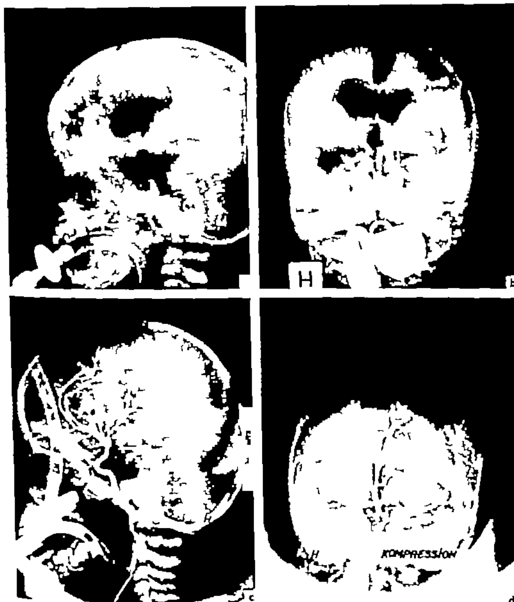


Fig. 1 (a) Lateral ventriculogram and (b) antero-posterior ventriculogram of 21-month-old boy with left-sided hemiplegia, showing dilated ventricular system, especially on the right side. There is small displacement towards the right and abundant surface air over the left hemisphere —none over the right ( ) and (d) Right carotid arteriogram showing displacement of the anterior cerebral artery towards the right. The middle cerebral vessels do not reach the inner surface of the skull on the right side. Both anterior cerebral arteries are filled, distended and elevated, indicating cortical atrophy

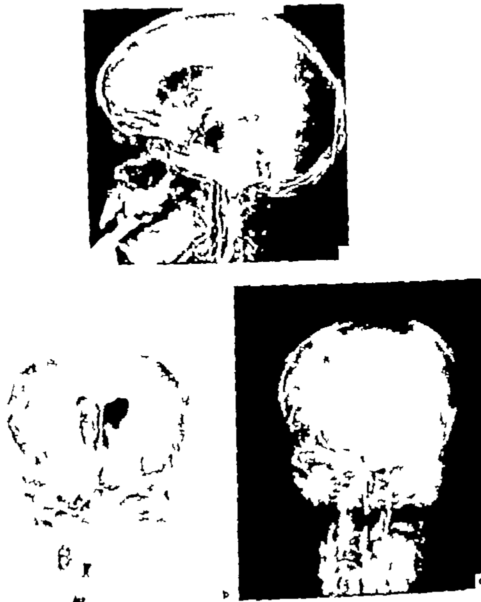


Fig. 2 ( ) and (b) Right carotid arteriography on an 11 year-old boy with left-sided hemiplegia, shows saccular aneur sm t the internal carotid artery site of division. ( ) Shows the same case immediately following operation with ttached Cushing' clips. There is no filling of anterior cerebral artery

The relation between *right sided* spastic h mipleglia and the arteriographic findings may be seen in Table 4 All three children with normal arteriographic findings also had normal encephalograms. The following report describes the findings in one of the two patients with an abnormal angiogram

A 12 year-old boy had severe clinical signs consisting of right-sided spastic paresis with hyperkinesia of the limbs from the first

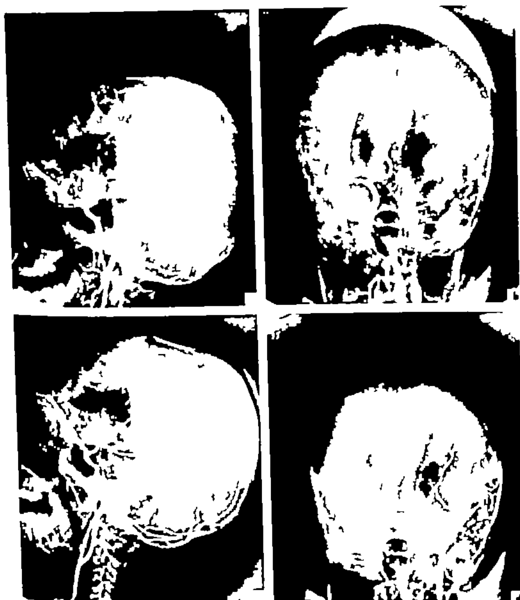


Fig. 3. ( ) and (b) Right carotid arteriography on 12 year-old boy with a right-sided spastic hemiplegia, performed one day after a lumbar encephalography show the anterior cerebral artery well developed and passing steeply up between he lateral entricles supplying both hemispheres. The preceding encephalography had shown dilatation of both lateral ventricles, primarily of the right, together with agenesis of the corpus callosum ( ) and (d) Left carotid arteriography shows no filling of the left nterior cerebral rtery

year of life. From the age of 9 focal seizures at times with loss of consciousness. Lumbar encephalography showed enlargement of the lateral ventricles, more marked on the left. The third ventricle showed changes typical of agenesis of the corpus callosum. Bilateral carotid arteriography disclosed a steep course of the anterior cerebral artery on the right. On the lateral view it was superimposed upon the lateral ventricles. On the antero-posterior views there was a compensatory development of the right anterior cerebral artery; the left one had probably never developed (Fig. 3).

(e) Patients with spastic tetraplegia

Another characteristic group among spastic children is tetraplegia. The present series included three children with spastic tetraplegia, one with spastic tetraplegia combined with choreo-ataxia and one combined with athetosis.

A 5-year-old boy was found to have epidural haematoma extending over a large part of the right hemisphere. He came to operation without preceding arteriography since there was vital indication for immediate neurosurgery. His acute condition improved immediately after the operation, but spastic tetraplegia persisted, and the boy showed slight mental impairment. After a repeated, less severe cranial injury carotid angiography was performed 3 months after the operation. This was normal, indicating that the tetraplegia could not be due to a recurrence of localized haematoma.

A 9-year-old boy had previously shown normal development. He was admitted to a department of neurosurgery after having been run over by a car, in a state of profound unconsciousness, with brain-stem seizures and spastic tetraplegia. He recovered only slowly and after bilateral arteriography supplemented by lumbar encephalography he was transferred to a department of general surgery because of a complicating fracture of the leg. The radiologic investigation had shown only slight enlargement of the ventricular system and no apparent extracerebral haematoma. From the surgical department the patient was transferred to a training department, but as he exhibited increasing lack of concentration, impairment of memory and unrest, he was referred to a paediatric department and right-sided carotid arteriography was repeated 8 months after the injury. The films now showed a subdural haematoma over the right hemisphere with additional suspicion of intracerebral haemorrhage (Fig. 4). Although not all the bilateral clinical manifestations could be accounted for by this haemorrhage, the repeated arteriography had at least detected a secondary pathology which could now be removed.

The arteriograms have given only a partial explanation of the tetraplegic symptom complex, but in one case they demonstrated and localized alarming haemorrhage and in another case afforded guidance for neurosurgery.

Summary and Conclusion

Our study of arteriograms and pneumoencephalograms from 24 children with cerebral palsy leads us to conclude that arteriography—however useful it may be in the acute and subacute stage of the disorder—offers very little more information than pneumoencephalography in the stationary stage of the disease. Ventricular dilatation may be suspected from the course of the arterial trunks and a shift may indicate atrophy of the involved hemisphere. Occasionally, however, indication of a vascular abnormality may be found, which would be undiagnosed by pneumoencephalography.

From some of our observations we are tempted to recommend that arteriography should be preferred to pneumoencephalography in patients with cerebral palsy in

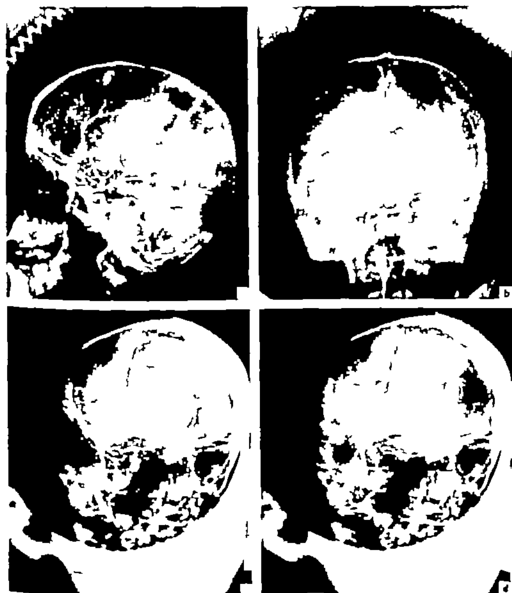


Fig. 4. Right carotid arteriography on 9 year-old boy with spastic tetraplegia, which he developed 3 months after skull trauma, shows large subdural haematoma over the right hemisphere together with pronounced displacement of the right anterior cerebral artery towards the left.

(1) cases of hemiplegia, especially with recurrent acute symptoms and exacerbations, (2) cases complicated by focal epileptic manifestations and (3) in persons in whom the symptoms of cerebral palsy may be of rather recent date perhaps not yet completely restored from their acute disease and in whom suspicion of overlooked pathology or complicating bleeding exists

References

1. BRANDT, S. and WESTERGAARD-NIELSEN, V.: Etiological factors in cerebral palsy and their correlation with various clinical entities. *Dan. Med. Bull.* 5: 47, 1958.
2. CHRISTENSEN, E. and BRANDT, S.: Recidiverende acut infantil hemiplegi forårsaget af dybt hemisphærangiom. *Nord. Med.* 62: 1834, 1959.
3. DENHOFF, E., HOLDEN, R. H. and SILVER, M. L.: Prognostic studies in children with cerebral palsy. *J.A.M.A.* 161: 781, 1956.
4. GREITZ, T.: A radiologic study of the brain circulation by rapid serial angiography of the carotid artery. *Acta Radiol. (Stockh.) Suppl.* 140, 1956.
5. HAMBY, W. B.: Rapport et discussions du premier congrès international des sciences neurologiques. Bruxelles 102, 1957.
6. PARAICZ, E. and SZENASY, J.: Über die Carotisangiographie im Kindesalter. *Acta Neurochir. (Wien)*, 7: 320, 1959.
7. POSER, CH. M. and TAVERAS, J. M.: Clinical aspect of cerebral angiography in children. *Pediatrics* 16: 73, 1955.
8. ROSVIG, S.: The pathology and surgical treatment of complicated cerebral palsy. *Arch. Pediat.* 75: 1, 1958.
9. SALOMONSEN, L., EEK, S. and SKATVEDT, M.: Pneumoencephalographic findings in cerebral palsy, epilepsy and oligophrenia. *Ann. Paediat. Fenn.* 3: 602, 1957.
10. SCHIEFER, W. and VETTER, K.: Das zerebrale Angiogram in den verschiedenen Altersstufen. *Zbl. Neurochir.* 17: 221, 1957.
11. TAVERAS, J. M. and POSER, CH. M.: Roentgenologic aspects of cerebral angiography in children. *Amer. J. Roentgenol.* 82: 371, 1959.

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Respiratory Studies in Children

III. Variability of the Lung Volumes in Healthy Children

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In a previous paper (3) the variability of the functional residual capacity (V_{FRC}) one of the components of th lung volume has been described and discussed.

The purposes of this work are to define the variability of the values of the other lung volumes in healthy children and to compare the variability with similar volumes in adults, since in many pulmonary diseases determination of the lung volumes is used not only for the diagnosis but also for longitudinal survey and to estimate efficiency of therapeutic procedures (6). It therefore seem useful to know exactly the limits of the day to-day physiological variation before drawing conclusions.

Definitions and Nomenclature

The nomenclature is that proposed by a committee f respiratory clinical physiologist headed by Pappenheimer (5) (Fig. 1)

1 The vital capacity (V_{VC}) was measured

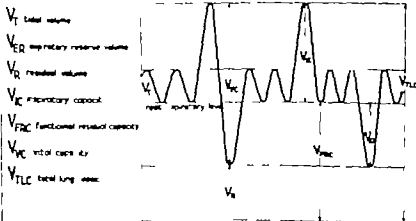


Fig 1

TABLE 1 *Lung volumes in healthy adults Time interval between the first (I) and the second (II) assay 4 days between the second and the third (III) 6 days See Fig 1 for symbols All the volumes are ml BTPS*

Subject no.	Standing height, cm	I	II	III	I	II	III
			V_{FRC}			V_{IC}	
1	18_	2387	3173	2831	5_14	5005	6029
	162	1640	2034	189	3318	3169	3190
3	182	_884	3157	3110	4630	4390	3315
4	163	2120	1 09	1714	3081	3061	2923
5	159	1659	1547	1870	31 1	3279	3329
6	156	1801	1714	1900	2607	2765	2847
7	174	3130	292_	2779	3900	3763	4069
8	158	2318	2373	2276	489	_858	2664
9	160	1765	1444	1707	2607	2480	2328
10	1 6	28_4	3183	3069	3850	4198	3959
			V_{VC}			V_{ER}	
1		6300	6201	6412	1146	1106	1353
2		4763	4110	4304	930	850	1163
3		6129	6010	5335	1499	1620	1640
4		4187	3990	383	1109	909	909
5		3537	3793	3990	711	814	731
6		3516	3555	3694	909	790	1047
7		5400	5412	5531	1600	1659	1482
8		3822	3632	3632	1343	1304	1148
9		3437	3279	3397	830	790	869
10		6063	6781	6123	2133	2175	173
			V_{R}			V_{TLC}	
1		1241	1977	1448	601	7179	8060
2		890	1106	1054	5158	5318	5336
3		1385	1537	1570	7514	7547	6823
4		1014	800	803	5201	4550	4837
5		948	1033	1319	4780	4826	4509
6		1022	924	853	4523	4470	4347
7		1350	1264	1317	6950	6678	6848
8		974	1080	1230	4807	4901	5063
9		932	654	833	4309	3833	4230
10		1491	1310	1190	6574	7591	6019

as the sum of the greatest inspiratory capacity (V_{IC}) and the greatest expiratory reserve volume (V_{ER}) chosen from three attempts at maximum inspiration followed by a maximum expiration.

In a previous paper (4) 64 records of vital capacity collected in 28 healthy and diseased children were critically analysed The conclusions were that it is practically impossible to systematically obt in the V_{VC} beginning with a deep expiration, and furthermore it is equally difficult to get three similar consecutive records. The latter is a requirement of many authors who have studied adults (1 5). As a result of this analysis, V_{VC} in children is calculated by adding the largest V_{IC} and the largest V_{ER}, chosen anywhere on the spirogram

2. The functional residual capacity (V_{FRC}) represents the volume of air which is left in the lung after a normal expiration during quiet breathing

TABLE .. *Lung volumes in healthy girls Four-hour i terval between the two italicized results Between these and the third four-day i terval*

Subject no.	Standing height, cm	I	II V_{FRC}	III	I	II V_{IC}	III
1	147	*1249*	*1133*	1320	*2014*	*2053*	21 0
2	160	*1115*	*1031*	125	*1817*	*2113*	1913
3	165	1788	*1851*	*1642*	133	*2054*	*2090*
4	104	*656*	*630*	6 4	*672*	*635*	849
5	145	*1009*	*1446*	1065	*2291*	*2109*	1935
6	164	1747	*1900*	*2013*	.347	*2706*	*2900*
	163	2003	*2095*	*2117*	2271	*1733*	*1817*
8	163	*1156*	*1320*	128	*2193*	*20 4*	2212
9	160	*22.3*	*2167*	180	*2504*	*3160*	2955
10	158	1389	*1219*	*1327*	23 3	*2211*	2870
11	129	*714*	*7 0*	730	*1330*	*1310*	1323
			V_{VC}			V_{ER}	
1		*2646*	*2407*	2565	*632*	*5 4*	415
		2626	*2646*	410	*469*	*653*	495
3		3239	*2544*	*2580*	1103	*790*	*760*
4		*864*	*968*	1007	*192*	*336*	136
5		*3338*	*3156*	3105	*1047*	*104*	1960
6		3990	*3674*	*3712*	1443	*968*	*752*
7		3377	*2544*	*3121*	1108	*1104*	*1304*
8		*3042*	*3002*	3002	*849*	*978*	790
9		*3910*	*4230*	4097	*1106*	*1006*	114
10		3436	*3041*	*3374*	1061	*829*	*554*
11		*1712*	*1700*	1738	*440*	*360*	418
			V_R			V_{TLC}	
1		*617*	*569*	905	*3263*	*3176*	3190
2		*236*	*478*	5	*2932*	*3144*	3167
3		683	*1091*	*85*	3922	*3935*	*3732*
4		*458*	*294*	516	*1322*	*1162*	16 3
5		*56*	*399*	405	*2900*	*3455*	3600
6		304	*932*	*1263*	4294	*4606*	*4975*
7		987	*993*	*1113*	4304	*4538*	*4 34*
8		*307*	*342*	49	*3349*	*3344*	3499
9		*1217*	1101	1033	*6127*	6327	5133
10		328	*420*	*773*	3704	*3461*	*4147*
11		274	410	321	1994	2110	2030

3. The residual volume (V_R) is calculated as the difference between V_{FRC} and V_{ER}.

4. The total lung capacity (V_{TLC}) is the sum of V and V_{VC}.

Methods

1. With the nose losed by a nose-clip, the subject breathes through mouthpiece into spirographic system, without valves, but with fan-type pump. The system is similar t that of Engström *et al.* (2): the differences between the two have been described in a previous paper (3).

2. A closed circuit with helium as the test gas was used for the determination of the functional residual cpacity (V_{FRC}) (3).

3. All the alues were corrected to body temperature ambient barometric pressure satured with water vapor (BTPS).

TABLE 3 *Lung volumes in healthy boys Four-day interval between the first (I) and the second (II) assay Six-day interval between the second and the third (III)*

Subject no.	Standing height, cm	I	II	III	I	II	III
			V_{FRC}			V_{IC}	
1	150	1549	1650	1545	2172	2445	2232
2	125	861	863	874	1560	1620	1341
3	140	1222	1626	1210	1560	1343	1490
4	143	1177	1156	1118	1716	1867	2015
5	149	1336	1482	1537	2205	2105	1757
6	171	470	430	494	3279	3418	3520
7	[illegible]	2310	2432	2350	2034	2250	2034
8	131	1137	1052	1016	1224	1305	1340
9	142	1246	1259	1251	2030	1890	2014
10	140	930	884	926	1 97	1936	1894
11	137	1163	1238	1066	2032	1933	2231
12	136	1049	1040	1034	1797	1910	2073
			V_{VC}			V_{ER}	
1		3156	3195	3141	983	750	800
2		1916	1965	1976	356	335	433
3		225_	2094	1955	672	751	475
4		27 6	2607	2469	1010	750	434
5		3035	2937	666	830	832	909
6		4622	4700	4995	1343	1282	1475
		3360	3090	304_	1306	830	963
8		1935	1817	1693	711	512	315
9		2622	404	2606	672	514	502
10		499	2489	2331	672	533	433
11		3002	2804	3121	970	851	379
12		2627	2464	2328	830	584	453
			V_R			V_{TLC}	
1		566	900	736	3722	4095	3877
2		503	528	439	24 1	2483	2418
3		651	375	735	2803	2460	2690
4		167	405	604	2893	3012	3133
5		726	650	828	3761	3587	3294
6		1127	1148	1019	5749	5848	6014
7		1004	603	1392	4364	3683	4434
8		426	540	701	2361	2357	2309
9		676	745	659	3298	3149	3245
10		258	351	491	2727	2840	2822
11		193	407	216	3196	3311	2237
12		219	496	583	2846	2950	3111

Material

1 Sixty-nine determinations were obtained while in the sitting position on 23 apparently healthy children, ranging in age from 6 to 15 years. Three assays were made on each patient with an interval of some hours to several days. The V_{FRC} of each assay equals the mean of duplicate determinations.

2 Thirty determinations were obtained on 10 apparently healthy young adults (students, nurses, etc.) with the same procedure as on children for comparison of the physiological variation

TABLE 4 *Mean values of the lung volumes in healthy adults boys and girls For details see Tables 1 to 3 The values are mean value (M) standard error (E) and standard deviation (S.D)*

		Adults (10 cases)			Boys (12 cases)			Girl (11 cases)		
		M.	E.	S.D	M.	E.	S.D.	M.	E.	S.D
V_{VC}	I	4704	±366	1162	2810	±211	730	2921	±281	932
	II	4646	±376	1188	2712	±219	760	2820	±269	891
	III	4647	±306	966	2710	±247	857	2829	±264	867
V_{IR}	I	3462	±288	911	1956	±146	507	2056	±181	600
	II	3456	±265	838	2004	±158	547	2040	±201	666
	III	3408	±252	796	2016	±160	563	2006	±198	657
V_{ER}	I	1223	±136	431	635	± 83	288	893	±107	355
	II	1190	±143	453	708	± 70	244	80	± 86	285
	III	1238	±132	418	684	± 98	333	734	±113	374
V_T	I	1146	± 79	250	544	±100	317	523	± 99	312
	II	1174	±119	376	639	± 97	320	593	± 69	228
	III	1123	± 87	275	767	± 83	303	657	± 65	204
V_{TLC}	I	5749	±402	1270	3345	±277	950	3476	±338	1098
	II	5719	±436	1378	3307	±276	956	3551	±458	1519
	III	5699	±393	1241	3399	±290	1006	3604	±328	1090

Results

The values for V_{FR} have been presented and discussed in a previous paper (3) The error of the method (standard deviation of the differences between duplicate determinations, divided by the square root of two) expressed in per cent of the corresponding V_{FRC} is 9 5 % for adults and boys and 3.0 % for girls.

There was no statistical difference in the mean values of V_{FRC} in each of the three assays for each subject. Since no differences in mean values were found, the physiological variation may be calculated

Taking only the assays performed at four-day intervals, the physiological variation is 190 ml for the adults 52 ml for the boys and 84 ml for the girls

When expressed in per cent of the mean of the first V_{FRC} in each group this variation is 7.5 3 7 and 5.8 %, respectively

The values for V_{VC} are given in Table 4 The individual differences ($V_{VC}I - V_{VC}II$) and ($V_{VC}II - V_{VC}III$) and mean differences were calculated. The first minus the second V_{VC} for both boys and girls at four-day intervals is probably significant. No other statistical differences were found

The values of physiological variation, excluding the two groups where statistical differences in means exist, are shown in Table 5.

TABLE 5 *Physiological variations of the vital capacity in healthy adults boys and girls*

Time interval	Adults (10 cases)	Boys (12 cases)	Girls (7 cases)
4 hours	—	—	93 ml
4 days	8 ml	—	93 ml
6 days	190 ml	123 ml	—

Standard deviation of the individual differences, $\sigma = \sqrt{S(x - \bar{x})^2/(n - 1)}$ Physiological variation $\sigma/\sqrt{2}$.

TABLE 6 *Percentage day-to-day physiological variation of V_{TC} (see text)*

	Time interval	V_{TC} mean ml	Variation in ml	Variation in %
Adults	4 days	4704	87	1.85
Adults	6 days	4704	190	4.03
Boys	6 days	271	133	4.9
Girls	4 days	2820	[illegible]	3.3

The physiological variation expressed in per cent of the first mean value of V_{TC} for adults and of the second mean value of V_{TC} for boys and girls (because the first mean value is significantly higher than the second and the third ones) is shown in Table 6

The values for V_{TC} are shown in Table 4 Neither the mean values for the first the second and the third assay nor the individual differences ($V_{TC} - V_{TC}$II and V_{TC}II $- V_{TC}$III) were statistically significant

The time interval between the assays varied from 4 hours to 6 days (Tables 1 to 3) Th physiological variation during four days was 109 ml for adults, 110 ml for boys and 179 ml for girls expressed in per cent of the mean first V_{TC} assay in each group, this is 3 1 5 0 and 8 8 % respectively

Thus the physiological variation for the three groups is about 6% (3 to 9 %)

The mean V_{ER} *values* (Table 4) for the three assays do not differ in the adults. The values of the first assay in the boys and in the girls are perhaps significantly higher than the values of the second and the third assay One of these differences (mean differences tested against zero) is significant the first V_{ER} is larger than the second V_{ER} at a four-day interval in boys ($P = 0\ 007$)

The physiological variation for each group (excluding groups with six cases or less) is shown in Table 7 and in per cent of the first mean value of V_{ER} for the adults and the girls and of the second mean value of V_{ER} for the boys in Table 8.

TABLE 7 *Physiological variation of the ex piratory volume in healthy adults boys and girls*

Time interval	Adults (10 cases)	Girls (7 cases)	Boys (12 cases)
4 hours	—	112 ml	—
4 days	77 ml	106 ml	—
6 days	114 ml	—	117 ml

TABLE 8. *Physiological variations for V_{ER} expressed in per cent*

	Time interval	Variation in ml	V_{ER} mean ml	Variation in %
Adults	4 days	77	1223	6.3
Adults	6 days	114	1223	9.3
Boys	6 days	117	706	16.6
Girls	4 days	106	895	11.8

TABLE 9 *Day-to-day physiological variations of the lung volumes in healthy adults boys and girls expressed in percent of the corresponding first assay*

	V_{FRC}	V_{VC}	V_{I}	V_{ER}	V_{R}	V_{TLC}
Adults	7.6	1.8	3.1	6.3	19	4.2
Boys	3 7	4.9	8.6	16 5	27	5.7
Girls	4.8	3 3	5.8	11.8	22	4.6

The physiological variation is from 6 to 17%.

The mean V_R values (Table 4) for each group did not differ significantly Taking only the assays with four-day intervals, the physiological variation was 216 ml for the adults, 145 ml for the boys and 150 ml for the girls, expressed in percent of the first V_R values the variation is 10 27 and 22% respectively

The V_{TLC} values (Table 4) also did not differ significantly The physiological variation during four days i 229 ml for the adults, 191 ml for the boys and 165 ml for the girls.

This percentage is 5 5 7 and 4.5% respectively

Discussion

Analysis of the individual differences and the mean differences reveals the first assay of V_{VC} is significantly higher than the second for both girl and boys and the first assay of V_{ER} i ignificantly higher than the second for th boys One could expect the reverse result if there were any influence of training on the values. It seemed to the observers that the children were not more trained but less enthusiastic after the first assay

The determination of V_{VC} V_{IC} nd V_{ER} (nd the subsequent calculat on of V and V_{TLC}) depend upon the peration of the subject In contrast, determination of V_{FRC} does not demand any effort from the child. The largest differences observed were for V_{ER} and V_R (V_{FRC} minus V_{ER}) The latter difference is primarily due to large variation of V_{ER} (Table 9)

The physiological variation of the total lung capacity (V_{TLC}) is about 5%. It depend on the variation of both V_{IC} and V_{FRC} ($V_{FRC}+V_{IC}=V_{TLC}$) To appreciate the magnitude of the variation of V_{TLC}, one ha to remember that the error of the method for the determination of V_{FRC} i 2.5 , for adults and boys 3% for girls, and that the value for V_{IC} requires the cooperation of the subject

Significant differences in the physiological variation of certain of the lung volumes between children and adults were demonstrated for V_{VC}, V_{IC} and V_{ER}

The day to-day variation in those volumes whi h demand the cooperation of the subject l lesser for the adult than for th children

Surprisingly the variation f V_{FRC} was higher in adults than in children The explanation i not clear and further study is needed

Summary

1 The day-to-day physiological variations of the l ng volumes were studied in healthy boys and girl The result were

mEq/day these infants were getting about three times their normal intake of P. On the 7th day urine was collected quantitatively for 24 hours and the pH, titratable acidity, ammonia and phosphorus were determined in it. The results were compared with those collected and described by McCance & Widdowson (26). Eight further babies were given the same phosphate mixture on the 5th, 6th and 7th day of their lives, but on the 7th day instead of collecting a specimen of urine covering a period of 24 hours, 2.8 mEq of NH_4Cl/kg were administered after a preliminary sample of urine and blood had been collected, and the 8 hours experiment already described was then carried out in exactly the same way.

Eight healthy adults were investigated in a comparable manner. After the collection of a preliminary timed, urine sample covering a period of 2 hours, blood was taken under paraffin by vein puncture. Four of the adults were given about 1.4 mEq/kg (54 mEq/m² body surface) of NH_4Cl and four others took the same amount of $CaCl_2$ diluted in water. It was originally intended to give the adults and the infants equal doses per kilogram of body weight, but 2.8 mEq/kg produced nausea and vomiting in the adults. The dosage was therefore equalised per square metre of body surface. Specimens of urine were collected under toluene every two hours for eight hours and a second vein puncture was performed four hours after giving the acidifying drug. Some months later the same eight adults were given 48 mEq of P per day in divided doses at a pH of 7.4 for 48 hours and on the second day the ammonium chloride test was carried out in exactly the same way as before.

Babies seemed to tolerate these doses of NH_4Cl and $CaCl_2$ equally well. Neither adults nor newborns suffered any ill effects during the experiment.

The timed specimens of urine passed by the babies during the 8 hours after the acidifying salt had been administered were split up and divided according to their volumes and times into 4 specimens each representing a period of 2 hours. These were separately analysed and so was the timed specimen passed before the drug was given.

The measurement of pH was made with B.D.H. capillators and the results have been expressed in terms of H ion concentrations so that they could be averaged and treated statistically. Titratable acid was determined by titrating the urine with 0.1 N or 0.01 N NaOH to pH 7.4 with phenol red as indicator. Ammonia was estimated by Conway's method (3); chlorides both in sera and urine by the method of Schales & Schales (37). For phosphorus the method described by Hawk, Oser & Summerson (16) was used. Urinary and serum Na and K were determined with an E.E.L. flame photometer after suitable dilutions. Serum bicarbonate was determined by Conway's microdiffusion method (3) in No. 2A units using 0.1 ml of serum. The figures for phosphate in mEq were arrived at by taking the valency for phosphate as 1.8. The averages given in the tables are all the means of the findings in 8 subjects, and they are followed by their standard deviations.

The basis of comparison

All those who have worked on the renal function of newborn animals have had to decide upon how their results are to be compared with those of adults (2, 7, 23). Most but not all (42) of those who have worked on human subjects have made their comparison per square metre of body surface while those who have worked on animals have used body weight. McCance & Widdowson (21) suggested that for some purposes body water would be the most correct basis to employ. This gives results close to those for body weight. Since the method employed may affect the conclusions to be drawn, it is most important to use the one which is likely to give results of most value for the purpose in hand. In the present investigation the doses were administered per square metre for the reasons already given. Some of the results are set out in Table 1 in four ways to show how very different they can be made to appear by varying the basis

TABLE 5 *Day-to-day physiological variation of the lung volumes in healthy adults boys and girls expressed in percent of the corresponding first assay*

	V_{FRC}	V_{VC}	V_{IC}	V_{ER}	V_{R}	V_{TLC}
Adults	7.5	1.8	2.1	6.2	19	3.–
Boys	3 7	4.9	5 6	16.5	27	5.7
Girls	5.6	3 3	5.8	11.8	23	4 5

The physiological variation is from 6 to 17 %.

The mean V_R values (Table 4) for each group did not differ significantly Taking only the assays with four-day intervals the physiological variation was [illegible] ml for the adults 145 ml for the boys and 120 ml for the girls expressed in percent of the first V_R values the variation is 19 27 and [illegible] % respectively

The V_{TLC} values (Table 4) also did not differ significantly The physiological variation during four days is 220 ml for the adults, 191 ml for the boys and 155 ml for the girls.

This percentage is 5.2, 5 7 and 4 5 % respectively

Discussion

Analyses of the individual differences and the mean differences reveals the first assay of V_V is significantly higher than the second for both girls and boys, and the first assay of V_{ER} is significantly higher than the second for the boys One could expect the reverse result if there were any influence of training on th val ues. It seemed t the observers that the hil dren were not more trained but less enthusiastic after the first assay

The determination of V V_{IC} and V_{ER} (and the subsequent calculation of V_R and V_{TLC}) depend upon the cooperation of the ubject In contrast determination of V_{FRC} does not demand any effort from the child The largest differences observed were for V_{ER} and V_R (V_{FRC} minus V_{ER}) The latter difference is primarily due to large variations of V_{ER} (Table 9)

The physiological variation of the total lung capacity (V_{TLC}) is about 5 %. It depends on the variation of both V_I and V_{FRC} ($V_{FRC}+V_{IC}=V_{TLC}$) To appreciate the magnitude of the variation of V_{TLC} one has to remember that the error of the method for the determination of V_{FR} is 2.5 % for adults and boys 3 % for girls, and that the value for V_{IC} requires the cooperation of the subject

Significant differences in the physiological variation of certain of the lung volumes between children and adults were demonstrated for V_C V_{IC} and V_{ER}

The day to-day variation in those vol umes which demand the cooperation of the subject is lesser for the adults than for the children

Surprisingly the variation of V_{FRC} was higher in adults than in children. The ex planation is not clear and further study is needed

Summary

1 Th day to-day physiological variations of the lung volumes were studied in healthy boys and girl The results

compared with the ones obtained in healthy adults.

2. The most striking variation was observed for the values of expiratory reserve volume which evokes the cooperation of the children and for the values of residual volume (because its calculation is a function of V_{ER})

References

1 Carlier, J Exploration fonctionnelle respiratoire chez les malades mitraux. *Rev Méd Liège 13* 360 1958.

2. Engström I Karlberg, P and Kraepelien S.: Respiratory studies in children. I Lung volumes in healthy children, 6–14 years of age *Acta Paediat 46* 277 1956.

3. Geubelle, F and D Rudder, P Respiratory studies in children, II Functional residual capacity in healthy children. *Acta Paediat* in press.

4 — Etudes respiratoires chez l'enfant. I. La capacité vitale et ses composantes. *Acta Paediat Belg 13*, 233, 1959

5. Pappenheimer, J R, et al.: Standardization of definitions and symbols in respiratory physiology *Fed Proc 9* 602, 1950.

6. Strang L. B.: Abnormalities of ventilatory capacity in children with asthma and bronchiectasis. *Arch Dis Child 35*, 224, 1960

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Renal Aspects of Acid Base Control in the Newly Born

III Response to Acidifying Drugs

by N HATEMI¹ and R. A. McCANCE

The renal functions of adults and of newborn infants have been compared in various ways and it has been shown that in many respects the newborn kidney is immature and McCance & Widdowson (23 4 25) have demonstrated that the stability of the "milieu interieur" may be maintained mainly by virtue of the integration of food and growth with renal function. The rôle of adults kidneys in the control of the hydrogen ion concentration in the internal environment has been fairly well investigated in normal, experimental and pathological states (11 29 31 33 38 44), and some account of the natural excretion of acids and bases in the newborn period was given by Widdowson & McCance (43) and McCance & Widdowson (26) No acidifying drugs were given in the last two investigations.

Gordon *et al* (12) and Fomon *et al* (10) gave six and eight infants respectively ammonium chloride in divided doses for some days and studied the effects. The infants were of different ages, there were no adult controls and the results do not strictly apply to the newborn period. Ruben *et al* (36) made somewhat similar experiments on premature infants 28 days old The aim of this present study has been to compare the responses of adults and newborn infants to the administration of single doses of acidifying drugs

Material and Methods

Twenty four seven day old male babies were studied. The first sample of urine passed in the morning was discarded in each case and the specimens passed for the next four hours were collected under toluene All the urine was obtained and preserved as described by McCance & Widdowson (22), except that each specimen was exactly timed and saved separately After this preliminary period of 4 hours, four of the babies were given 2.8 mEq/kg of NH_4Cl (54 mEq/m² of body surface) in a single dose and four the same amount of $CaCl_2$. The salt was dissolved in breast milk and given at 1 p.m. immediately before breast feeding. Blood samples were taken by heel prick under paraffin oil before and about 4 hours after giving the acidifying drug Urine was collected for 8 hours, or as near to this time as the spontaneous voidings of the infants allowed. Eight infants were given 7 mEq of P per day in divided doses on the 5th, 6th and 7th days of their lives. It was given as a mixture of M/7.5 Na_2HPO_4 and M/7.5 KH_2PO_4 at pH 7.4 Since the daily intake of P in their mothers milk was of the order of 2.5

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mEq/day these infant were getting about three times their normal intake of P On the 7th day urine was collected quantitatively for 4 hours and the pH, titratable acidity ammonia and phosphorus were determined in it. The results were compared with those collected and described by McCance & Widdowson (*6) Eight further babies were given the same phosphate mixture on the 5th, 6th and th da of their lives, but on the 7th day instead of collecting a specimen of urine covering a period of 4 hours, 2.8 mEq of NH Cl kg were administered after a preliminary sample of urine and blood had been collected, and the 8 hours experiment already described was then carried out in exactly the same way

Eight healthy adult were investigated in a comparable manner After the collection of a preliminary timed, urine sample covering a period of hours, blood was t ken under paraffin by vein puncture Four of the adult were given about 1 4 mEq kg (54 mEq m body surface) of NH Cl and four others took the same amount of CaCl diluted in water It was originally intended to gi the adult and the infant equal doses per kilogram of body w ight but 8 mEq kg produced nausea and vomiting in the adults. The dosage w therefore equalised per square metre of body surface Specimens of urine were collected under toluene every two hours for eight hours and a second vein puncture was performed four hours after giving the acidifying drug Some months later the same eight adult were given 48 mEq of P per day n divided doses at a pH of 4 for 48 hours and on the second day the ammonium hloride test w carried out in exactly the same wa before

Babies seemed t toler t these doses of NH Cl and CaCl equall well Neither adults nor newborns suffered an ll effects during the experiment

The timed specimens of urine passed by the babies during the 8 hours after the acid fying salt had been administered were split up and divided according to their ol mes and times into 4 specimens ea h representing period of hours These were separately analysed and so was the timed specimen passed before the drug was given

The measurement of pH was made with B D H capillators and the results have been expressed in terms of H ion concentrations so that they could be averaged and treated statistically Titratable acid was determined by titrating the urine with 0 1 N or 0 01 N N OH to pH 4 with phenol red as indicator Ammonia was estimated by Conway's method (3) chlorides both in sera and urine by the method of Schales & Schales (37) For phosphorus the method described by Hawk, Oser & Summerson (15) was used. Urinary and serum Na and K were determined with an E.E.L. flame photometer after suitable dil tions. Serum bicarbonate was determined by Conway microdiffusion method (3) in No 2A units using 0 1 ml of serum The figures for phosphate in mEq were arrived at by taking the valency for phosphate as 1 8. The averages given in the tables are all the means of the findings in 8 subjects, and they are followed by their standard deviations.

The basis of comparison

All those who have worked on the renal function of newborn animals have had t decide upon how their result are to be compared with those of adults (–, 7 27 25). Most but not all (42) of those who have worked on human subjects ha e made their comparison per square metre of body surface while those who have worked on animals ha e used body weight McCance & Widdowson (21) suggested that for some purposes body water would be the most correct basis to employ This gives results close to those for body weight Since the method employed may affect the conclusions t be drawn it is most important t use the one which is likely to gi e results of most value for the purpose in hand In the present investigation the doses were administered per square metre for the reasons already given. Some of the results are set out in Table 1 in four ways t show how very different they can be made to ppear by varying the basis

TABLE 1 *Th renal responses of fants to ammon um chloride compared with those of ad lts d d ff rent way*

Measurement compared	m^2	kg of body wt	basal metabolic rate	as or of	of potential H ions hloride administered
	Basis of comparison				
Excretion of					
Ammonium salt	18	4_	0		1
Titratable acid	9	23	11		11
Total acid	14	33	18		1
Chloride	3	6	3		39

N.B. 1 The dose in all cases was 34 mEq NH_4Cl m
_ Only the figures for the infants are given in the T ble and each has been calculated on the assumption that the adult value was 100
3. The basal metabolic rates of infants and of adults were taken t be 16_ and 14 0 kcal h respectively—Smith (40), Suzuki (41).

of comparison. It is interesting and perhaps important to not how closely the result ex pressed per unit of basal metabolism agree with the only direct evidence vailable namely the percentage of the dose excreted. Most of the comparisons in this paper have been made on basis of surface area, but body weight has also been used t present certain aspects. Ultimately it is very much t be hoped that standards of normality will be available for human infants and children of all ages This will bviat the need for so many comparisons with adults in clinical work, but it is unlikely that such standards of reference will ever be available for all the species of experimental animals likely to be employed.

Results

1 *The changes n the serum*

Table _ shows the average serum chloride and the total CO in adult and infants before and 3 t 4 hours after the administration of ammoni m chloride The total CO was initially a little lower in the infants than in the adult The ffect of the drug wa lso somewhat greater infant and it may be ssumed that the stimulus to exret hydrogen ions should have been at lea t great or greater

* *The correction of the acidosis*

Table 3 shows the effect of giving breast fed human infants 54 mEq of NH Cl or $CaCl_2$/m as a single dose and Table 4 what happened when the same treatment was meted out to normal adults. (The dose for adult wa much smaller than for infant / kg of body weight.) The infant (a) dif fered considerably more among them selves but raised the average H ion con centration ([H] 10 ⁷) of their urine less rapidly and to a considerably smaller ex tent than adults; (b) had little titratal le ed in their preliminary urine and only doubled it after the acidifying drug whereas the adults had more titratable acid initially and increased it by four times a the [H] 10^{-7} of the urine rose ( ) excreted nearly as much ammonia a the adult before the drug was given but in creased the rate of xcretion much less afterwards. Consequently the overall ex cretion of H ion wa less than that of the adult in the preliminary specimens of urine and the response to the acidifying drug much less (d) had only very mall amount f I in the urine at all times ( ) excreted the Cl on less rapidly at ll

TABLE 2 *Effect of giving 54 mEq/m² NH₄Cl or CaCl₂ on the chlorides and total CO₂ in the plasma of adults and of breast-fed infants 7 days old with and without neutral phosphate*

Subjects	Chloride mEq/l Before	Chloride mEq/l After	Total CO_2, mEq/l Before	Total CO_2, mEq/l After
Adults	106 ± 2.0	109 ± 2.3	29 ± 1.6	26 ± 2.2
Infant on breast milk alone	109 ± 2.2	112 ± 4.1	24 ± _1	19 ± 3 3
Infants on breast milk plus phosphat	109 ± 3.3	115 ± 2.8	25 ± 1 4	21 ± 2.5

times; (*f*) excreted less sodium and potassium per hour than the adults before the drug was given and at all times afterwards.

Since the amount of titratable acid which can be excreted in the urine is well known to be linked to the amount of buffer substances and particularly phosphates being excreted simultaneously (20 42) it was thought that the absence of phosphates from the infants urine at all times might severely limit their capacity to excrete hydrogen ions in the form of titratable acid and consequently their ability to respond to an acidifying drug Table 5 shows the effect of giving 7 mEq of P 6.2 mEq of Na and 0.8 mEq of K a day to breast fed infants. The results for normal infants fed on breast milk and cows milk have been previously reported (26) The administration of phosphates in this way made little or no difference to the $[H^+] \times 10^{-7}$ or to the excretion of ammonia over a period of 24 hours, but it raised the excretion of phosphates from 0 75 to 56.9 mEq/kg/24 hr and the titratable acidity by nearly four times. The infants fed on cows milk preparations had more acid urines and excreted more phosphates, titratable acid and ammonia than either of the two other groups.

Table 6 shows the effect of giving NH_4Cl as a single dose on the seventh day of life to babies who were being given 7 mEq of P per day on the 5th 6th and 7th days after birth. The results should be compared with those of the babies who

TABLE 3 *The effect of giving breast fed infants aged 7 days 54 mEq of NH Cl or CaCl₂/m² as a single dose.*

All the results except those for [H] 10⁻ are given as mEq/m /hr
The figures for P = amount excreted in mg 1 8/31

	Before giving NH_4Cl or $CaCl_2$	Hours after giving NH Cl or $CaCl_2$: 0–2	2–4	4–6	6–8
[H] 10^{-7}	6 2 ± 10.5	12.7 ± 11.6	28 0 ± 28.8	40.5 ± 45.1	60.4 ± 54.8
Titratable acid	0 06 ± 0 05	0.09 ± 0.06	0.12 ± 0.10	0.14 ± 0.10	0.12 ± 0.06
Ammonia	0 40 ± 0 13	0.47 ± 0.17	0.57 ± 0 14	0.59 ± 0.11	0.52 ± 0.17
Ammonia + titratable acid	0.46 ± 0.16	0.55 ± 0.13	0.69 ± 0.22	0.73 ± 0.17	0.65 ± 0.22
Chloride	1.37 ± 0.49	1.90 ± 0.57	2.79 ± 2.53	3.22 ± 1 73	2 85 ± 1.20
P	0.06 ± 0.13	0.06 ± 0.14	0.06 ± 0 16	0.04 ± 0.12	0.04 ± 0.10
N	1 18 ± 0.65	1.88 ± 0.57	2.87 ± 2.13	2.66 ± 1 73	2.11 ± 1 12
K	0 48 ± 0 20	0.51 ± 0.31	0.82 ± 0.60	0.79 ± 0.43	0.73 ± 0.43

TABLE 4 *The effect of giving normal adults 5 mEq of NH_4Cl or $CaCl_2/m^2$ as a single dose*

All the results except those for [H] 10⁻⁸ are expressed as mEq/m /hr
The figures for P = amount excreted in mg 1.8/31

	Before giving NH_4Cl or $CaCl_2$	Hours after giving NH_4Cl or $CaCl_2$ 0–2	2–4	4–6	6–8
[H] 10^{-7}	[illegible] ± [illegible]	51[illegible] ± 50.9	13 ± [illegible]	14[illegible] ± 25.5	150 ± [illegible]
Titratable acid	0.26 ± 0.23	0.48 ± 0.31	0.82 ± 0.22	1 01 ± 0.20	0.96 ± 0.22
Ammonia	0.56 ± 0.23	1.29 ± 0.16	1.33 ± 0.29	1.20 ± 0.32	1.24 ± 0.35
Ammonia + titratable acid	0.82 ± 0.45	1.75 ± 0.33	[illegible]25 ± 0.32	2.31 ± 0.36	2.20 ± 0.60
Chloride	2.71 ± 1 70	8.03 ± 4.90	11.88 ± 4.51	8.06 ± 2.5[illegible]	[illegible]87 ± 2.06
P	0 45 ± 0.26	0 43 ± 0.23	0.72 ± 0.25	0 [illegible]7 ± 0.41	0.75 ± 0.27
Na	2.21 ± 1.37	6.16 ± 2.86	6.56 ± 3.68	6.11 ± 2.19	5.55 ± 2.31
K	1.34 ± 0.60	1.[illegible]7 ± 0.70	[illegible]39 ± 0.58	2.03 ± 0.60	1.90 ± 0.60

TABLE 5. *Effect of giving 7 mEq P/day in solution at pH 7.4 on urinary pH and on the rates of excretion of inorganic phosphate titratable acid and ammonia in breast fed infants 7 days old.*

Treatment	[H] 10^{-9}	Phosphate µEq/kg/hr	Titratable acid µEq/kg/hr	Ammonia µEq/kg/hr	Titratable acid + ammonia µEq/kg/hr
Breast milk alone*	11.8 ± 18.7	0.[illegible]5 ± 0.46	6.3 ± 4.[illegible]	22.5 ± 6[illegible]	29.8
Breast milk plus phosphate	11.9 ± 17 4	56.9 ± 12.4	22 ± 11 4	21.5 ± 4 9	43.5 ± 13.9
Cow milk*	16.2 ± [illegible] 4	93 ± 22.2	42 ± 10.8	30 ± 5.4	72

Data from McCance & Widdowson (26)

TABLE 6 *The effect of giving NH_4Cl as before to breast fed infants on the 7th day of their lives The babies were also being given mEq of P/day on this and on the two previous days*

All the results except those for [H] 10⁻⁹ are given as mEq/m²/hr
The figures for P = the amount excreted in mg 1.8/31

	Before giving NH_4Cl	Hours after giving NH_4Cl 0–	2–4	4–6	6–8
[H] 10^{-9}	1 40 ± 2.46	1 70 + 1 11	3.31 ± 1.56	11.2 ± 9 0	15.3 ± 11.6
Titratable acid	0 19 ± 0.14	0.27 ± 0 1	0.42 ± 0.22	0.47 ± 0[illegible]1	0.51 ± 0[illegible]4
Ammonia	0[illegible]4 ± 0.09	0.42 ± 0.17	0.57 ± 0.22	0.52 ± 0[illegible]1	0 42 ± 0.19
Ammonia + titratable acid	0.43 ± 0 20	0.69 ± 0.32	0.99 ± 0.30	0.99 ± 0.36	0.94 ± 0.31
Chloride	0.81 ± 0 [illegible]4	1.58 ± 0.45	2.17 ± 0.90	2.12 ± 1.16	1.95 ± 1.06
P	0 82 ± 0.27	1.02 ± 0.32	0.85 ± 0 42	0.80 ± 0.33	0.69 ± 0 52
Na	1 28 ± 0.40	2.31 ± 0.66	2.30 ± 1.11	2.02 ± 1.21	1.80 ± 1 15
K	0.80 ± 0.25	0 68 ± 0.26	0.61 ± 0.29	0.65 ± 0.27	0 74 ± 0.40

TABLE 7. *The effect of giving NH_4Cl to adults as before who were also taking 48 mEq of P in divided doses on the same and on the previous day*

All the results except those for $[H^+] \times 10^{-7}$ are given as mEq/m²/hr
The figures P = the amount excreted in mg × 1·6/31

	Before giving NH_4Cl	Hours after giving NH_4Cl			
		0–	2–4	4–6	6–8
$[H^+] \times 10^{-7}$	7 6 ± 8.75	39.0 ± 33.1	148 ± 88.9	65.2 ± 40.[illegible]	62.4 ± 42.1
Titratable acid	0.28 ± 0.22	0.87 ± 0.38	1.84 ± 0.43	1.99 ± 0.42	1.85 ± 0.80
Ammonia	1.59 ± 0.14	1.44 ± 0.53	1.37 ± 0.23	1.41 ± 0.29	1.28 ± 0.33
Ammonia + titratable acid	0.97 ± 0.37	[illegible]41 ± 0.52	3.[illegible]1 ± 0.61	3.40 ± 0.63	3.26 ± 0.73
Chloride	4.13 ± 1.50	8.04 ± [illegible]	8.84 ± 2.61	8.51 ± 1.86	8.71 ± 1.30
P	0.86 ± 0.4	1.43 ± 1.10	2.44 ± 0.95	3.00 ± 0.78	2.82 ± 1.41
Na	3.84 ± 1.37	6.40 ± 2.31	6.92 ± 2.29	6.89 ± 0.81	6.26 ± 1.71
K	1.54 ± 0.80	1.98 ± 0.65	[illegible]29 ± 0.68	3.07 ± 1.25	3.33 ± 1.09

were not given phosphates (Table 3) and with those of the adults in Tables 4 and 7. Table 7 shows the effect of giving 48 mEq of phosphorus per day for two days as a neutral solution to adults and 54 mEq of NH_4Cl/m² on the second of these two days.

The babies given phosphates plus NH_4Cl (*a*) excreted 0.03 to 1.07 mEq of P/m²/hr throughout the test, (*b*) raised the $[H^+] \times 10^{-7}$ of their urines no more, indeed rather less, than those not given phosphates, but (*c*) excreted more titratable acid before and considerably more after being given the ammonium chloride, (*d*) excreted less ammonia before and slightly less after the acidifying drug. Owing to the increased excretion of titratable acid, however, these infants got rid of more of the H ions generated by the drug than those not taking phosphates in spite of the fact that the $[H^+] \times 10^{-7}$ of their urines was never quite so high but (*e*) they excreted less chloride ions; (*f*) their excretion of sodium and potassium was about the same.

Although the dose was relatively much smaller, giving the phosphates to the adults increased by [illegible]–4 times the amounts of P being excreted in the urine. It made no appreciable difference to the rate at which these adults were able to raise the $[H^+] \times 10^{-7}$ of their urine after NH_4Cl and consequently it greatly increased the excretion of titratable acid. It made little or no difference to the rate of excretion of ammonia at any time but, as in the babies, it materially increased the elimination of the H ions generated by the drug and, also as in the babies, it reduced the excretion of chloride ions.

Table 8 gives four ways in which the differences between the ability of the adult and the newly born to correct the acidosis may be summarised. It shows in order the maximum rate of excretion of titratable acid + ammonia and the maximum H ion "clearance" index, both measured over [illegible]-hour periods. The H ion clearance is the most sensitive method hitherto devised of assessing the ability of a person to maintain systemic neutrality. It was worked out by Elkinton & McCance (Elkinton, Huth, Webster & McCance (9)). The "clearance index" is defined as the mEq of titratable acid + ammonia excreted per min per 1.73 m² × plasma bicarbonate

TABLE 8 *The greatest rate of excretion of H ions + ½H₂ and the highest H ion clearance index (both measured over two hour periods) and the percentage of the dose of potential H ions and of chloride excreted within 8 hours by adults and infants*

Measurement	Infants	Adult
Maximum rate of excretion of H ions mEq/1.73 m²/hr without phosphate pretreatment	1 09	4.00
Maximum H ion clearance index without phosphate pretreatment	0.23	1 70
Maximum rate of excretion of H ions mEq/1 73 m²/hr after the administration of phosphate	1.70	6.00
Maximum H ion clearance index after the administration of phosphate	0.67	2.50
Percentage of the H ion excreted in 8 hours		
(a) Without administering phosphates	3.4 ± 1 8	19.7 ± 6.9
(b) After administering phosphates	8.8 ± .8	31 0 ± 6 3
Percentage of the Cl ions excreted in 8 hours		
( ) Without administering phosphates	3..0 ± 1 .3	79.9 ± 33.7
(b) After administering phosphates	21.2 ± 12.7	53.5 ± 23.4

(in mEq) T ble 8 also shows the percentage of the dose of potential H ions generated by the drug excreted at each age within 8 hours and the percentage of the chloride ions. In each case the rate of excretion before the drug was given has been taken as the base line and deducted from the rates found over the next 8 hours.

In all four parameters the infants made a poor showing as compared with adults, but it must be recalled that had the dosages and results been expressed per kg of body weight the infants would have received a larger dose than the adults but they would have made a much better showing

3 *The total response to ammon um chloride*

Th average cation-anion relationship of the urine of the unmedicated breast fed infant is shown in Fig 1a. It is known from the work of Slater (30) on 18 other breast fed infants, that on the 7th day of their lives infants absorb about 95 µEq/kg/hr of potassium, 100 of sodium, 107 of chloride and 49 of phosphorus and it may be taken that the babies in the present investigation were absorbing about the same The serum tissue renal relationships were causing the excretion of 145 µEq/kg/hr of (measured) cations mostly balanced by chlorides and there were only traces of phosphates in the urine The administration of the phosphates increased the intake of total cations by 84 µEq/kg/hr for the previous 48 hours as shown in Fig. 1b. This was not a large addition, for the food had been providing about 105 µEq/kg/hr and the addition provoked almost no change in the x cretion of total cations (see Fig 1c) The rate of excretion of phosphates went up—if only to about half that of the additional intake—and this led to the readjustment between titratable acid and ammonia which has already been noted—but since there was only a trifling increase in the total excretion of cations the rat of excretion of chlorides necessarily fell This may be regarded as an example of the ion antagonism discussed by Rapoport & W

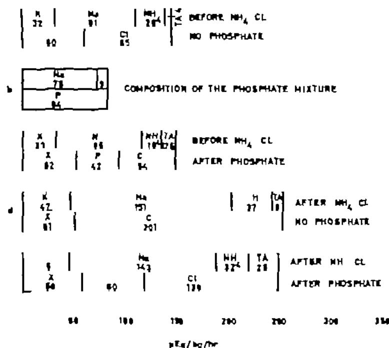


Fig. 1 The effect of giving NH Cl without and with pretreatment with neutral phosphates on the cation anion balances of the urine in infants. The measurements shown in blocks a and refer t the preliminary periods, and those in blocks d and cover the 3 hours after the drugs had been given. b shows the composition of the neutral phosphates administered. (– undetermined anions.)

(34) but the results of Bank & Schwartz (1) should also be considered. These last authors found that the infusion of neutral sodium phosphate to sodium depleted dogs led to the preferential excretion of the phosphate much titratable acid and urines with a very low pH.

Giving the ammonium chloride without the preliminary administration of phosphates created an internal acidosis (see Table 2) Fig 1*d* shows that this raised the excretion of titratable acid + ammonia from 32 to 45 µEq/kg/hr This may not seem much and it was only a fraction of the total response (compare Fig 1*a*) which included a large rise in the excretion of Na and a smaller one in that of K. The rate of cation excretion rose from 145 to 250 µEq/kg/hr and the whole of this was balanced by chlorides.

The exhibition of the neutral phosphates made little difference to the internal acidosis created by the ammonium chloride (Table 2) nor did it make any difference to the total cation response of the kidney (Fig 1*d* and *e*) The phosphates, however considerably increased the rate of excretion of titratable acid + ammonia and produced a readjustment between them as it had done when no NH_4Cl had been given (Fig 1*d* and *e*, and *a* and *c*) The rate of excretion of phosphates was considerable although again less than the additional intake (Fig. 1*a* and *c*) and again since giving the phosphates did not increase the rate of excretion of cations,

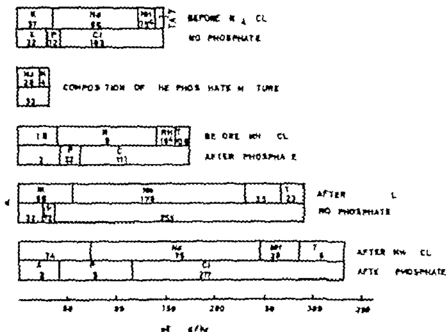


Fig 2. The effect of giving NH_4Cl without and with pretreatment with neutral phosphates on the cation-anion balances of the urine in adults. The measurements shown in blocks a and refer to the preliminary periods, and those in blocks d and cover the 8 hours after the drugs had been given. b shows the composition of the neutral phosphates administered. (= undetermined anions)

the rate of excretion of chlorides necessarily fell.

The average anion-cation relationships of the urine of the normal adults are shown in Fig. *a Without having been given phosphates or ammonium chloride the adults xcreted 148 µEq of measured cations per hour which was close to the rate in infants, although there were differences in the ammonia-titratable acid relationships These cations were balanced by rather more chlorides than in the infants and by phosphates

It cannot be assumed the rates of intake of sodium chloride potassium and phosphorus were equal to the measured rates of excretion for the latter are subject to considerable diurnal variations but all the experiments were done at the same time of day to minimise this variable. The administration of the phosphates increased the intake of total cations considerably less than in infants (see Fig 16) Neverthe less it raised the excretion of titratable acid and of sodium and almost doubled the excretion of phosphorus (Fig c).

The internal acidosis provoked by ammonium chloride without phosphates raised the excretion of titratable acid and ammonia, increased the excretion of potassium and considerably increased that of sodium This last was almost entirely balanced by chloride and there was no increase in the xcretion of phosphorus (Fig a and d) This wa a materially greater total response however than the

ammonium chloride produced in the babies (compare Fig 1 d and 2 d) After giving the neutral phosphates to the adults as well as ammonium chloride there was the usual increase in the excretion of total cations (compare Fig 2 c and 2 e) The increased excretion of phosphorus was roughly the same as in the infants and although the excretion of chlorides more than doubled, the increase was not so great as it had been when phosphates had not been given (Fig 2 a and d and c and e) Thus the total response to the internal acidosis in infants was fundamentally the same as in adults and consisted of an increase in the output of titratable acid and to some extent of ammonia but also a great increase in the output of sodium and chloride The administration and excretion of the phosphates did not make any difference to the output of total cations by the babies and not a great one to the output in adults and consequently the increased excretion of phosphates led to the excretion of less chlorides, at both ages.

Discussion

The administration of neutral phosphates to infants days old resulted in a considerable increase in the elimination of free H ions, without any significant decrease in the output of ammonium ions It is possible that even before any acidifying drug was administered the capacity of the infants to eliminate H ions at the existing concentration of total CO_2 in the plasma was limited to some extent by the absence of enough urinary buffers This at all events is the assumption usually made about patients with renal acidosis (18 44) It may be instructive to regard the findings in the serum and urine of the newborn infant as originating in the same way as those in a case of incomplete renal acidosis. Since however it was pointed out by Schiess *et al.* (38) that the administration of neutral phosphate may have the above effect in normal adults the difference between them and the newborn must be regarded as a relative rather than an absolute one

These present experiments have differed in two respects from any of the previous ones carried out on human infants. Thus (1) they were based on the effects of administering single doses of the acidifying drugs and (2) the infants were breast fed and therefore excreting little or no inorganic phosphates. The present experiments therefore were more like those of Cort & McCance (4) on newborn puppies than those of Gordon *et al* (12) or of Fomon *et al* (10) on infants.

After the administration of the acidifying drugs the average $[H^+] \times 10^{-}$ of the infants urines rose less quickly and completely than it did in adults. In other words the infants were unable within the time limits of these experiments to establish as great an H ion gradient between plasma and urine. This is probably true of other newborn animals but puppies have been found more able than human infants (4) and piglets less able (14). Without enquiring too closely into the mechanisms involved the results again recall those found in cases of renal acidosis. The infants studied by Gordon *et al.* (1) excreted 24 hour urines with pH values as low as 4 6 after ammonium chloride but they had been longer out of the uterus than the present series, their serum bi-

carbonates became much more abnormal, they were being fed on cows milk and the drugs were given for several days. Even in those experiments however some of the infants did not lower the pH of their urine (1., 17) as much as adults would have done

The lack of phosphates in the urine of newborn infants severely limited the excretion of titratable acid at all times. However even after the administration of neutral phosphates plus NH_4Cl the average rates of titratable acid excretion did not reach adult levels, partly because the infants failed to raise the [H+] 10^{-} of their urines as much as the adults did. These results, therefore confirmed a prediction of Dean & McCance (6) The results after administering phosphates as well as NH Cl were not unlike those obtained by Pitts & Lotspeich (3.) on acidotic dogs.

Most previous experiments, including those of Cort & McCance (4) (see also Robinson (35)) have turned on the ability of the newborn subject to excrete ammonium ions and the general conclusion has been that the defect relative to adults may be considerable This is probably correct so far as puppies are concerned. The present experiments have brought out new features in man. Owing to the paucity of buffer substances in the urine the *human infant before birth and, if* breast-fed, after birth, must rely upon ammonia for the greater part of the H ion elimination in the urine (20) McCance & Widdowson (20) found that at 7 days of age the normal rate of xcretion of m monia per kg of body weight was equal to that of adults and the present results after NH Cl bears this out if they are looked at per kg of body weight instead of per unit of surface area. It is possible that the ammonia production of these infants was reasonably good and being utilised almost to capacity before the drugs were given, and that this accounts in part for the small increase afterwards. From the data of other investigators, however it seems that the ability to increase the excretion of ammonia during an acidosis is not as well developed in breast fed infants as it is in those fed on cows milk (10) Possibly the availability of ammonia precursors (30) or the development of the necessary enzymatic processes (5, 16) may also be limiting factors

To judge by these experiments the new born breast fed infant is in a state of mild acidosis as indicated by its serum bicarbonate and its response to the administration of a solution of neutral phosphates. The excretion of H ions generated by the administration of ammonium or calcium chloride is limited by (*a*) the failure to raise the [H] $\times 10^{-7}$ of the urine so rapidly or so fully as adults and (*b*) the paucity of urinary buffers. Even when this was completely rectified the rate of H ion excretion (in all forms) was still only two-thirds that of adults and certainly not so good at corresponding levels of plasma total CO " or following a similar fall from the normal steady state This all *suggests that the ceiling for the production* of H ions by the infants kidney is lower than it is in adults.

Cows milk provides the infant with more acid to excret than breast milk (probably mostly sulphates) but also provides buffer substances in the shape of phosphates with which to excret it as titratable acidity In healthy newborn infant

the two effects roughly cancel each other out but the infant on cows milk is usually slightly the more acidosed than the one on breast milk (13 19) Owing to the continued ingestion of cows milk, however the kidneys of infants so fed may become able to excrete H ions better than those of breast-fed infants for as early as the third month their hydrogen ion clearance indices may reach the adult levels (mean ? 07 range 1 4–3 4) (9) after the administration of acidifying drugs while those of breast fed infants of a similar age calculated from the data of Fomon *et al* (10) are still very much lower with a mean of 1 01 and a range of 0.34–1 40

It is not usual to consider what has been termed the total response to ammonium chloride although others have spoken rather teleologically of the increase in ammonia production sparing the excretion of fixed base It is suggested that the excretion of sodium and potassium i part of the response of the organism to a fall in the pH of its plasma and tissues and that it represents the excretion of sodium and potassium which have been rendered temporarily redundant by the operation of the buffering systems in all parts of the body (9) The pattern of all these responses may be found to differ from the newborn animal of one species to that of another according to their states of development at birth (14)

Summary

1 When a solution of sodium and potassium phosphates at pH 4 were administered to normal brea t fed infants aged 5 6 and 7 days they excreted considerably more H ion (n the form of t tratable acid).

2 After the administration of a single dose of an acidifying drug normal breast fed infants " days old (*a*) raised the $[H^+] \times 10^{-7}$ of their urine less rapidly and completely than adults (*b*) excreted less of the H ions generated by the drugs but (*c*) excreted almost as much ammonia/kg of body weight

3 The administration of neutral phosphates to adults (*a*) made no difference to the rate and extent of the rise of $[H^+] \times 10^{-7}$ after ammonium chloride (*b*) increased the excretion of H ions in the form of titratable acid without reducing the excretion of ammonium ions. After giving phosphates to the infants they (*a*) maintained the $[H^+] \times 10^{-}$ of the urine at a lower level, (*b*) increased the excretion of free H ions but (*c*) reduced the excretion of ammonia and consequently (*d*) did not increase the excretion of free H ions + ammonia so much as adults

4 The normal breast fed infant appears to be handicapped in dealing with a sudden acidosis by (*a*) an inability to raise the $[H^+] \times 10^{-7}$ of the urine rapidly and completely (*b*) an absence of urinary buffers, (*c*) an overall failure to excrete H ions so rapidly as adults This may amount to a general deficiency on the part of the kidney to produce the necessary H ions to be excreted

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References

1 B NK, N., and SCHWARTZ, W B.: The influence of anion penetrating ability on urinary acidification and the excretion of titratable acid. *J Cli Invest, 39* 1616 1960.

— BARNETT H. L., and VESTERDAL, J The physiologic and clinical significance of immaturity of kidney function in young infants. *J Pediat, 42* 99 1953.

3. CONW Y E. J Microdiffusion Analysis and Volumetric Error 4th ed. London Crosby Lockwood & Son.

4 CORT J H., and MCCANCE, R. A.: The renal response of puppies to an acidosis. *J Physiol, 124* 358, 1954.

5. D VIES, B. M. A., and YUDKIN J Studies in biochemical adaptation. The origin of urinary ammonia as indicated by the effect of chronic acidosis and alkalosis on some renal enzymes in the rat. *Biochem J 46* 407 1952.

6. DEAN, R. F A., and MCCANCE, R. A. Phosphate clearances in infants and adults. *J Physiol 107* 182 1948.

7 EDELMANN C. M., JR., and BARNETT H. L.: Role of the kidney in water metabolism in young infants. *J Pediat, 56* 154, 1960

8. ELKINTON J R.: Whole body buffers in the regulation of acid base equilibrium. In Essays in Metabolism. Boston, Toronto: Little Brown & Co.

9 ELKINTON J R., HUTH, E. J WEBSTER, G. D JR., and MCCANCE, R. A.: The renal excretion of hydrogen ion in renal tubular acidosis I. Quantitative assessment of the response to ammonium chloride as an acid load. *Amer J Med 29* 554, 1960.

10. F NON S. J HARRIS, D. M and JENNEY R. L. Acidification of the urine by infants fed human milk and whole cow milk. *Pediatrics 23* 112, 1930

11. GILMAN A and BRAZE C P The role of the kidney in the regulation of acid-base metabolism. *Amer J Med 15* 765, 1953.

12. GORDON H. H., MCNAMARA, H., and BEN ARIS H R. The response f young infants t ingestion of ammonium chloride *Pediatrics, 2* 290, 1948.

13. GYÖRGY P KRAPPEN, F and KRUM, F Das Säurebasengleichgewicht im Blut mit besonderer Berücksichtigung des Kindesalters. I H ionenkonzentration und CO_2-gehalt *Z Kinderheilk. 41* 700 1926.

14. H TEKL N and MCCANCE, R. A The response of piglet t ammonium chloride T be published in J Ph siol

15. H WK P B OSER, B. L. and SUMMERSON W H Practical Physiological Chemistry 13th ed. London J & A. Churchill 1954.

16. HINES B. E., and MCCANCE, R. A.: Ammonia formation from glutamine by kidney slices from adult and newborn animals. *J Physiol, 124* 8 1948.

17 H YEMAN W S., PARMELEE, A. H., and GROSSMAN A. Mechanism of production of acidosis in premature infants by protein milk. *Amer J Dis Child, 75* 687 1948.

18. LATNER, A. L., and BURNARD, E. D Idiopathic hyperchloremic acidosis of infants (Nephrocalcinosa infantum). Observations on the site and nature of the lesion. *Quart J Med, 19* 285, 1950.

19 MARPLES, E., and LIPPARD V W Acid base balance of newborn infants. III. Influence of cow milk on the acid-base balance of the blood of newborn infants. *Amer J Dis Child, 45* 294 1933.

20 MCCANCE, R. A., and OW FINCH, M. A.: The titratable acidity pH ammonia and phosphates in the urines of very young infants. *Arch Dis Childh, 22* 200, 1947

21 MCC NCE, R. A., and WIDDOWSON E. M.: The correct physiological bases on which to compare infant and adult renal function. *Lancet II* 860, 1952.

— — Renal function before birth. *Proc Roy Soc B, 141* 488 1953.

23. — Metabolism, growth and renal function of piglets in the first days of life. *Arch Dis Childh, 133*, 373, 1956.

24 — New thoughts on renal fun tion the early days of life. *Brit Med Bull, 13* 3, 1957

25 — The response of the newborn piglet t an excess of potassium. *J Physiol 141* 88, 1958.

26. — Renal aspects of acid-base control in the newly born. I Natural development. *Acta Pediat, 49* 409 1960.

27 MCCANCE, R. A., and YOUNG, W F.: The secretion of urine by newborn infants. *J Physiol, 99* 265 1941

28. MERTOFF J Some aspects of renal structure and function concerned with regulation of body fluids. *Pediat Clin N Amer 6* No. 1 1959.

29. ORLOFF J The role of the kidney in the regulation of acid base balance *Yale J Biol Med, 29* 211 1956.

30 ORLOFF J and BERLINER, R. W The mechanism of the excretion of ammonia in the dog *J Cli Invest 35* 223, 1956.

31 PITTS, R. F Acid-base regulation by the kidneys. *Amer J Med, 9* 356, 19?

32. PITTS, R. F and LOTSPEICH, W D.: Factors governing the rate of excretion of titra-

table acid in the dog. *Amer J Physiol*, *147* 451 1946
33. Pitts, R. F., Lotspeich W. D. Schiess, W. A., and Ayer, J. L. The renal regulation of acid base balance in man. I. The nature of the mechanism for acidifying the urine. *J Clin Invest* *27* 48 1948.
34. Rapoport S., and West C. D. Ionic antagonism. Effect of various anions on chloride excretion during osmotic diuresis in the dog. *Amer J Physiol* *163* 668 1950
35. Robinson J. R.: Ammonia formation by surviving kidney slices without specific substrates. *J Physiol* *124* 1 1954.
36. Rubin B. L., Calcagno, P. L., Rubin M. L., and Weintraub, M. D.: Renal defense response to induced acidosis in premature infants (ammonia production and titratable acid excretion) *Amer J Dis Child*, *92* 513, 1956.
37. Schales, O. and Schales, S. S.: A simple and accurate method for the determination of chloride in biological fluids. *J Biol Chem* *140* 879 1941
38. Schiess, W. A., Ayer, J. L., Lotspeich W. D., and Pitts, R. F.: The renal regulation of acid-base balance in man. II. Factors affecting the excretion of titratable acid by the normal human subject. *J Clin Invest* *27* 57 1948
39. Slater, J. E.: Retentions of nitrogen and minerals by babies one week old. *Brit J Nutrit*, 1961
40. Smith, C. A.: The Physiology of the New-born Infant. 3rd ed. Oxford: Blackwell Scientific Publications. 1959.
41. Suzuki S. Basal metabolism in the Japanese population. *World Rev Nutrit & Dietetics* Chap. 5 105, 1960.
42. Weber, H.: Über die Beziehung zwischen Phosphat und Säureausscheidung im Harn bei gesunden und kranken Kindern. *Hel Paediat Acta*, *11*, 186, 1960.
43. Widdowson E. M., and McCance, R. A.: The development of acid-base control. *Ciba Foundation Colloquia on Ageing*, *4* 209 1958
44. Wrong O., and Davies, H. E. F. The excretion of acid in renal disease *Quart J Med* *28* 259 1959

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Preliminary Experiences with the Spitz Holter Ventriculo-Caval Shunt in the Treatment of Hydrocephalus[1]

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Since the beginning of the present century surgeons have tried to solve the problem of treating communicating hydrocephalus, but only recently have the results been promising. In the last few years a large number of cases have been operated on utilizing plastic tubing for drainage of cerebrospinal fluid (CSF) into the abdominal or pleural cavities or into the ureter. The attempts to drain this fluid directly into the circulating blood have been unsuccessful due to thrombosis. By using valves allowing only uni-directional flow of CSF into the blood the number of successful cases has increased. Such a valve designed by Spitz and Holter has been used in America and England with promising results. We have used this technique in five cases of progressive infantile hydrocephalus and in one case of intracranial hypertension due to diffuse melanosarcomatosis of the meninges. The preliminary results have been encouraging.

History

The *first* attempt to relieve hydrocephalus by operation was the simple drainage of CSF to the exterior of the skull but these patients soon died from infection. Drainage of CSF back to the blood was suggested by Gartner as early as 1895. In 1908 Payr (18) reported operations in three cases where the lateral ventricle was anastomosed to the superior sagittal sinus utilizing a free segment of vein. However all patients died within four months of the operation. Later Payr (1911) reported other ventriculo-venous shunt operations where connection between the lateral ventricle and the internal jugular vein was made. Excellent results were obtained in three out of eight patients. For a comprehensive review of the earlier operative methods in the treatment of hydrocephalus see Davidoff (1929).

Decreased production of CSF by excision of the choroid plexus was first suggested by Dandy in 1919 and it was further modified and improved by Hyndman (1946) and Scarff (1952). The latter reported twenty-five good results out of thirty-nine operated cases with observation times of more than six years.

Ingraham *et al.*, (1948) were the *first* to report the use of plastic tubing to drain CSF into the circulating blood of experimental animals. In every instance blood refluxed into the tube and clotted. They concluded that a valve allowing only uni-directional

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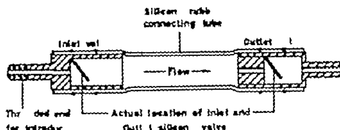


Fig. 1 Simplified drawing of Holter alve to illustrat the principles of construction.

flow of CSF into the blood would be necessary The f rst really promising clinical result were presented by Cone *et al* (1949) using lumbo-peritoneal shunts and Spitz & Koop (1952) reported seventy three per cent success with this technique Matson (1949) introduced the lumbo-ureteral shunt and of fourty-seven patients thirty-six good results were reported (15) The disad antages of the Matson procedure are the sacrifice of one kidney and the dangers of electrolyt losses and infection Another technique developed by Halom (1955), consists of shunting CSF from the lateral ventricle to the extradural space of the cervical and thoracic regions where large amounts of fluid can be absorbed In Scandinavia. Andersson & Bohm (1958) have reported their experience using the lumbo-peritoneal shunt technique and in their cases the t p of the catheter was connected to a specially constructed teflon button Of nine operated cases three showed good results.

The first use of a valve in ventriculo-venous shunts as suggested by the experiments of Ingraham *et al* was reported by Nulsen & Spitz (1952) They used a system consisting of a rubber subcutaneous pump connecting two ball valves This case wa operated on in 1949 and the shunt was functioning well at the time of thei initial report in 195. Further modifications i mprovements were made by Holter in 195 (Holter (1950), see also Mcnab (1958)) Another type of valve has been successfully used I P udenz *et al* (195) Here the flow controlling alve is situated in the tip of a catheter going down the superior vena cava to the right atrium.

The Holter valve

The Holter valve consists of a pair of fishmouth type valves connected in series by a short length of silicone rubber tubing This valve allows only uni directional flow of fluid and is designed to open at two different pressures of about ten and fifty mm of water respectively It is about 5 cm in length and 6 mm in diameter (Fig 1)

The valve system is placed under the scalp in the mastoid region. A proximal catheter penetrates the brain so that the tip will lie freely in the lateral ventricle Another catheter attached to the distal valve is tied into the internal jugular vein and the tip of this catheter should lie freely in the right atrium.

The valve mechanism lies directly under the scalp and by compressing the rubber cylinder connecting the two valves, fluid can be pumped at any time to confirm that the system is patent This is one of the great advantages of the Holter valve The site of a possible block can also be determined Compression of the valve mechanism without refilling denotes a block in the ventricle If the valve is not compressible there is obstruction on the venous side



Fig 2

Operative technique

A preoperative roentgenogram is taken in order to estimate the approximate length of the distal, cardiac catheter This length is measured from the point where the catheter is tied into the jugular vein down to 1 cm above the carina (junction of superior vena cava and right atrium) About 10 per cent is deducted due to magnification.

The child is placed on the operating table in the supine position with the head turned to the left exposing the right temporal region and stretching the right side of the neck (Fig *). A curved incision is made in th right posterior temporal region and the flap is retracted. A burr hole for the ventricular catheter placed *so that the valve does not tend dow* into the neck area A groove is made in the bone to keep the v lve in place The proximal, ventricular catheter i inserted into the lateral ventri le using a tylet An incision is made in the neck and the n ternal jugular vein exposed by blunt dissection through the sternomastoid muscle By the use of a special ntroducer the valve with its cardiac catheter already attached is passed through the cervical incision up under the skin to its position in the *groove in the mastoid region where it is* fixed with a steel wire The ventricular catheter is now attached to the proxim l valve of the pump The jugular vein is opened and the cardiac catheter passed down the vena cava. (Beware of air embolism!) The proper position of the tip of the catheter may be checked by a roentgenogram by fluoroscopy or by lec trocardiography (23) In the latter case a conducting wire is passed through the caval catheter so that its tip lies just bove the distal opening of the tubing This wire and one arm are connected to the electrocardiograph forming an ECG lead Maxi*mal size of the P-deflection indicates that* the tip of the catheter is situated near the centre of the uricle

At the site where the cardiac catheter passes into the jugular vein the catheter i divided and threaded ver a special plastic connector which can then be firmly tied into the jugular vein without risk of bstructing the catheter

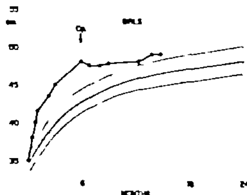


Fig 3 Case 1 Ordinate: head circumference in cm. Abscissa: age in months.

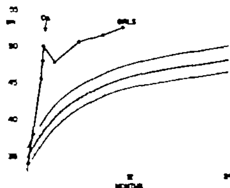


Fig. 4. Case 2. Ordinate- head circumference in cm. Abscissa: age in months.

Material and Methods

Our material comprises five cases of communicating hydrocephalus, two of which had been previously operated for a meningocele In one case an Arnold-Chiari malformation was diagnosed. All cases were examined by pre- and postoperative measurement of head circumference pneumography (postoperatively with small amounts of oxygen) recording of the ventricular fluid pressure (VFP) and mental and physical examination by pediatricians.

In the diagrams illustrating the increase in head circumference the shaded area represents the distribution in 80 per cent (between 10 and 90 per cent) of a study of white infants of North European descent living in or near Boston (24).

The VFP was recorded during 20 to 30 minutes using a technique described by Lundberg (13) In this technique the ventricle is punctured with the cannula connected to the pressure chamber of a strain gauge transducer Escape of fluid during the procedure is thus avoided. Care was taken to obtain records during periods, when the child was calm, if necessary during feeding

The last observations included in the follow-up were made in November 1959

Case Reports

Case 1 G M. S Born May 19 1958. Immediately after birth vomiting and rapidly increasing head circumference of unknown etiology (Fig 3). July 23 1958, encephalography· communicating hydrocephalus. November 10, 1958, operation. Spitz Holter ventriculo-caval shunt. No complications. Control examination August 10, 1959 pediatric examination, normal development. Lumbar encephalography slight increase in brain substance (measured at frontal lobe), VFP 2 to 5 mm Hg under pentothal anesthesia.

Case K. G Born September 19 1958. During first weeks of lif rapid increase in head circumference of unknown etiology (Fig 4) November 14 mean VFP about 20 mm Hg (Fig. 5). Encephalography November 17 1958 communicating hydrocephalus November 19 1958 Spitz Holter ventriculo-caval shunt Fever of unknown origin after a few weeks without local signs of infection. August 1959 lumbar encephalography and recording of VFP: increase in brain substance; mean VFP about 3 mm Hg (Fig 5). October 1959 intermittent signs of increased intracranial pressure October 1, 1959 reoperation. Free passage in both catheters, the distal catheter checked by injection of radio-opaqu material; valve functioning well. November 8, 1959 signs of acut increase of intracranial pressure. Reoperation: obstruction in proximal catheter relieved, catheter too long Postoperatively no complications, valve functioning well. Pediatric examination: normal development.

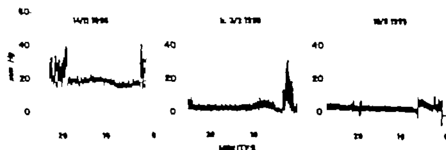


Fig. 5 Case 2. Ventricular fluid pressure before ( ) and fter (b,) shunt operation. Large deflections in Fig.a and b produced by crying (When the child was quiet the records always showed fairly even pressure level without any large fluctuations. Cf Lundberg (13).)

Case 3 A. J Born October 14, 1958. Born with a cervical meningocele and Arnold-Chiari malformation. October 14, 1958: excision of cervical meningocele No complications in the immediate postoperative period, but in the following weeks he showed increasing signs of hydrocephalus with rapid increase in head circumference (Fig. 6) November 1958, lumbar encephalography: communicating hydrocephalus. December 12, 1958, operation: Spitz Holter ventriculo-caval shunt. April 1959 increasing signs of septicemia, no signs of infection in the operative region; valve functioning well. Septicemia controlled with antibiotics. August 1959 pediatric examination: mental and motor development somewhat retarded Lumbar encephalography: increase in brain substance. VFP measurement normal.

Case 4 B J Born May 31 1958 At birth meningocele in the lumbo-sacral region without neurologic symptoms. Operation June 1958 excision of the meningocele After this operation rapidly increasing head circumference (Fig 7). Encephalography August 1958 hydrocephalus. Encephalography January 1959 showed further dilatation of the ventricles. January 20, 1959 operation. Spitz Holter ventriculo-caval shunt No complications. August 1959 control examination: pediatric examination, normal development; lumbar encephalography showed increase in brain substance 39 mm compared to 30 mm before operation (Fig. 8) Recording of the CSFP (lumbar needle) under pentothal anesthesia showed a mean pressure of about mm Hg

Case 5 M. F Born September 11 1958. Immediately after birth, rapid increase in

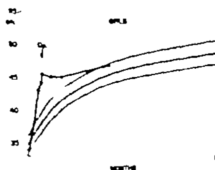


Fig 6 Case 3. Ordinate: head circumference in cm. Abscissa: age in months.

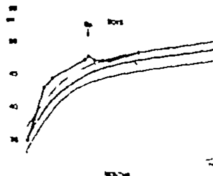


Fig. 7 Case 4. Ordinate: head circumference in cm. Abscissa, age in months





b

Fig 8 Case 4 ... (pneumo)encephalographs See text.

head circumference of unknown etiology (Fig 8). Encephalography March 1959- communicating hydrocephalus. Recording of the VFP mean pressure about 12 mm Hg. April *7 1959 operation Spitz Holter ventriculo-caval shunt. August 13, 1959, control examination pediatric examination mental development normal, motor development somewhat retarded; lumbar encephalography and VFP recording increase in brain substance; mean VFP about 5 mm Hg. September 30 1959- valve not functioning Reoperation required, September 30, 1959- examination of shunt showed the right jugular vein t be bstructed. November 5, 1959- ventriculo-venous shunt on the left side. No postoperative complications. Valve functioning well.

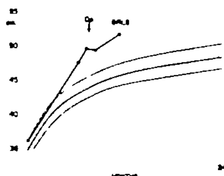


Fig 9 Case 3. Ordinate: head circumference in cm. Abscissa: age in months.

Comments

Among these five operated cases there were no postoperative deaths. Three reoperations were necessary In one case the obstruction was localized to the jugular vein and was probably due to thrombosis. This failure was most probably caused by the catheter being too short In one other patient two reoperations were necessary w thin a time of only two weeks both due to obstruction which by th pumping technique wa located t a site proximal t the valve At the first reoperation no block of the ventricular catheter was found At the second reoperation the passage through the ventricular cannula wa found t be obstructed. probably because the tip was embedded in brain substance In one case there were prolonged signs f sepsis (Staphylococcus albus) finally con trolled by ant biotics I this case there were no signs of nfection in the operative field and no signs f thrombosis obstruct ing the caval catheter At the end of the follow up time (November 1959) all valves were functioning well

The present valve was developed early in 1956 and has now been used in over five thousand cases all over the world (8) Spitz (23) has used the valve in over five hundred cases and he regards the results as very gratifying At the beginning reoperations were necessary in about thirty per cent but this figure has later been reduced to about ten per cent. Carrington ( ) ha recently presented a series of fifty patients where the H lter valve had been used. In forty three the valves were functioning well Ten reoperations were necessary and eight of these were due to obstruction of the caval catheter (throm bosis)

One of the main argument against this operation has been the factor of body growth. It has ho ever been estimated that the distance from mastoid to xiphoid increases only 6 cm in the first years of lif and the new silicone tubing has the capacity of 300 per cent stretch

In the treatment of hydrocephalus the fact must be considered that in about 40 per cent of the cases arrest will occur spon taneou ly (1*) In such cases a shunt may be of benefit even if it hould stop func tioning after the acut symptoms

subsided. The early diagnosis and treatment of infantile hydrocephalus is of great importance in view of the rapid reduction of brain substance that may result from increased intraventricular pressure

It is too early to evaluate fully the shunt operation with the Holter valve but the results so far are promising

Addendum

The Spitz–Holter procedure has also been used for palliative treatment of an adult patient with intracranial hypertension the origin of which was not diagnosed until post mortem

Case 6 Diffuse melanosarcomatosis of the meninges with intracranial hypertension

T S A a man, aged 44 was admitted to the Neurosurgical Department in Lund because of signs of severe intracranial hypertension His disease had started four months previously with headache vomiting, confusion and unsteadiness of gait Eventually he became more and more confused and agitated and he was repeatedly treated in a mental hospital. Three months after onset bilateral papilledema was found Encephalography showed some herniation of the cerebellar tonsils and moderate widening of the ventricles, but no signs of a space-occupying lesion There were no cells in the cerebrospinal fluid but the protein content was considerably increased. Vertebral angiography was normal.

Six months after onset he was admitted to the Neurological Department in Lund He was then completely confused and disorientated, hallucinated, agitated and restless Stiffness of the neck, paresis of the right abducens nerve and bilateral papilledema of 5 diopters was recorded He was admitted to the Neurosurgical Department because of increasing signs of intracranial hypertension

In addition to the above mentioned signs, he had attacks of impaired consciousness, increased agitation and stiffness of the neck and irregular respiration. Ventriculography showed the same state as at the previous encephalography *i.e.* no signs of any localized lesion and free passage of the gas from the ventricles to the subarachnoidal space. The patient was treated by repeated lumbar punctures, which relieved his symptoms of intracranial hypertension. The protein content of the cerebrospinal fluid was high (1000–2000 mg per cent). Later continuous drainage of the right lateral ventricle was instituted using a uni-directional valve. By this means the VFP was kept at a normal or slightly elevated level during a total of about four weeks. During the period the patient was less restless and agitated and there was no impairment of consciousness, but his mental state was otherwise unchanged. A slight regression of his papilledema was observed by the ophthalmologists The valve was sometimes closed and the VFP recorded for a short period (Figs. 10a and 10b).

The shunt operation was performed 4 weeks after admission to the Neurosurgical Department During the first days the pressure was low (Fig 10c) Three day postoperatively the pressure started to rise but could still be temporarily reduced by pumping the val (Fig 11a). Eight days after operation when the ventricular cannula was removed the VFP was still bove normal. At a control ventriculometry three weeks later the VFP was about 4 mm H and the same level was recorded 3½ mon postoperatively (Figs. 11b and 11). The v functioned well as shown in Fig. 12. A time the patient was still severely co and disorientated. His general co had deteriorated, he was permanent ridden and severely emaciated. As red with the preoperati e stat th edema had diminished but there protrusion of 2 diopters in the lef 4 diopters in the right. Some preserved but how much was ass assess. The protein content in the fluid was still high (about 200 The patient was transferred

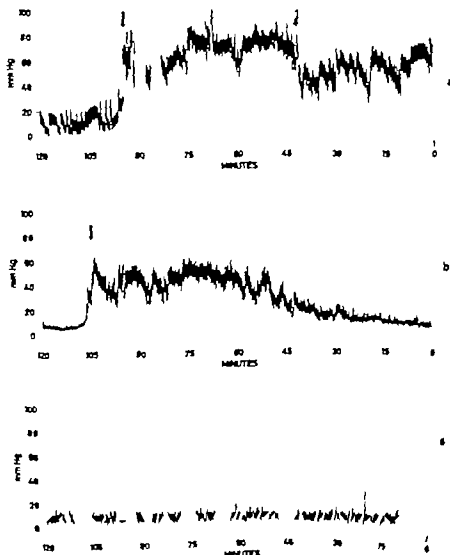


Fig. 10. Case 6 and b VFP before shun operation. Unfilled arrow: ... Arrow: Tapping of ventricular fluid VFP first da postop. The curves should be read from right to left

hospital where he died months later 6 months after the shunt operation. From the subsequent course of the disease it appeared that the val e was functioning until death

Postmortem examination

Examination of the ... meninges on the base of the brain ... seat of heavy ...

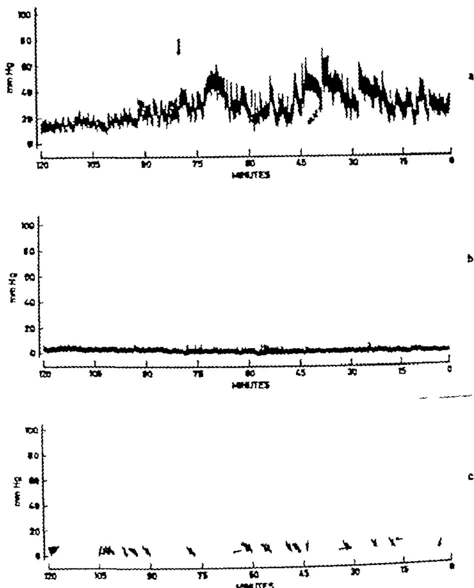


Fig 11 Case 6 VFP 3rd da post p Arrow indicates when valve is first compressed. b. VFP 3 eeks postop VFP 3½ month postop The curves should be read from right to left.

was moderat w lening of the th rd and fourth entricles but no ppreciabl le ing and no disconfiguration of the l teral n tricles. Histological xam nat on t th me ninges t the base of the brain l on the floor of the third entricle showed or of melanotic tumor cell There were m of inflammatory reaction or infection of the walls or the lateral ventricles or the meninges of the frontal lobes.

Comments This case provided us with valuable experience It showed that in tracranial hypertension may be relieved

for a long time by the Spitz–Holter shunt even if the ventricles are small and the fluid pathways patent The procedure may thus be useful in selected cases of so called pseudotumor cerebri and replace subtemporal decompression which has been widely used to relieve headache and preserve vision in such cases, but probably with limited benefit The ventriculo-venous shunt may also be used as a palliative measure in selected patients with inoperable brain tumours and in patients where the intracranial hypertension is not relieved by excising a tumor provided that *shunting between the ventricles and* the subarachnoidal space is not expected to be of benefit.

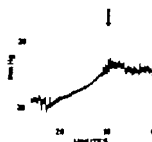


Fig 12 Cases 6. VFP 3½ months postoperatively Arrow indicates when valve is first compressed.

Summary

A preliminary report is given of 5 cases of infantile hydrocephalus where ventriculo-venous shunt operations using the Spitz–Holter technique were performed. There were no postoperative death In all cases pre- nd postoperative measurements of head circumference pneumography of the ventricular system recordings of the ventricular fluid pressure and examinations by pediatricians with regard to mental and physical development were performed. Th follow up time ranged between 6 and 1 months. Three reoperations of the shunt were necessary in two patients, after which all valves were functioning well. In all 5 cases interruption of the rapid increase in the head *circumference occurred* and a *low ventri*cular fluid pressure was found postoperatively In 4 cases post perative pneumography suggested an increase in brain bulk. Normal mental development was found in 4 of the cases

In addition a brief report is given of a case of increased intracranial pressure due to diffuse melanosarcomatosis of the meninges in an adult In this case a Spitz–Holter shunt operation reduced a preoperatively high and widely fluctuating ventricular fluid pressure to a low even level during an observation time of 6 months It is suggested that the Spitz–Holter operation may be of benefit in cases of severe intracranial hypertension where removal of an i tracranial tumour or an intracranial hunt operation does not give relief

References

1 ANDERSSON H and BOSTE, E Om infantil h drocephalus och dess behandling *Svensk Läkartid* 41 3051 1939
2 CARRINGTON K. W Ven riculo enous shun using the Holter alv trea men of hydrocephalus *J Mich Med Soc* 58 373–376, 393 1958.
3 COVE, W A LEWIS, R. D and J CKSON I J Pres meeting of *Amer Coll Phys*, Montreal, Canada, Sept 23, 1949
4 D ND W E Experimental hydrocephalus *A Surg 70* 129, 1919.
5 D NOTT L Trea men of hydrocephalus *Arch Surg* 19 1737 1929.

6 GIRTNER: Cited by K usch.

7 HA AM, B JIMENEZ, A., and ROMAN, F.: Drainage of the cerebrosp nal fluid int the spinal epidural space new techni que for the treatment f hydrocephalus. *Acta Neurochir (Wien* 1 3 1965

8 HOLTER, J W Personal communication, 1959

9 HYND LA O R H lehus. *J Neuro-surg, J* 426–443

10 ING AHAM, F D 1 D D ALEX ANDER J F s, R. P Studies in the treat t f perimental hydro-ceph lus. r *Exp N urol* 1 3– 1 1

11 K v n l ll ung des Hydroce-p h nder *Arch Kli Chir*

12 (unic nunication t 9th m n, Brit Paediat Ass, 1951

13 L n J on inuous recording and o l of ula fluid pressure in neu surgical practice *Acta Psychiat Scand S pl 119* openhagen, 1960

14 MATSON D D A new operation for the treatment of communicating hydrocephalus Report f case secondary t generalized meningitis. *J Neurosurg 6* 38– 47 1949

15 M TSON D D Ventriculo-ureterostomy *J Neurosurg 8* 398–404 1951

16. MACNAB G H. Hydrocephalus of infancy Recent Advances in Paediatrics. Ed. by Douglas Gairdner J & A. Churchill, Ltd London, 1958

17 NULSEN F E., and SPITZ, E. B.: Treatment of hydrocephalus by direct shunt from ventricle t jugular vein. Surgical Forum. *Amer Coll Surg Philadelphia*, 2 399–403, 195

18. PAYR, E. Drainage der Hirnventrikel mittelst frei transplantierter Blutgefässe. Bemerkungen über Hydrocephalus. *Arch Klin Chir 87* 801–885, 1908.

19 PAYR, E.: Über Ventrikeldrainage bei Hydrocephalus. *Verh Deutsch Ges Chir 40*, 515–535, 1911

20 PUDENZ, R. H., RUSSEL, F E., HURD, A H and SHELDEN C. H.: Ventriculo-auriculostomy Technique for shunting cerebrospinal fluid into the right auricle. *J Neurosurg 14* 171–179, 1957

21 SCARFF J E. Nonobstructive hydrocephalus. *J Neurosurg 9*–164, 1952.

22. SPITZ, E., and KOOP C.: Forum meeting *Amer Coll Surg* Sept. 1952.

23. SPITZ, E.: Personal communication, 1958, 1960

24 STUART H. C., and STEVENSON, S. S.: Physical growth and development In Nelson: "*Textbook of Pediatrics*" Philadelphia & London, 1954.

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Symposium on Haemolytic Disorders in Childhood

From the Departments of Paediatrics and Clinical Chemistry University Hospital Uppsala Sweden

Contents

Bo Vahlquist[1] Introduction

During the last week of September 1960 the Swedish Paediatric Society had the pleasure of the company of 20 members of the British Paediatric Association as its guests. The group visited Gothenburg, Stockholm, and Uppsala. Meetings were arranged at each of these university clinics the guests reading papers and the hosts arranging symposia. The subject of the Uppsala symposium was *Haemolytic Disorders in Childhood* and the choice was influenced by the fact that this subject has for many years been one of the main research topics of the Department of Paediatrics in Uppsala.

There is probably no field within haematology which has developed so rapidly in the last few decades as that relating to the haemolytic disorders. As a matter of fact, so many new laboratory methods have been introduced, and so many new observations made that it is becoming increasingly difficult for the individual physician to keep abreast with them all. The papers now published do not embrace the iso-immunization disorders a field large enough to deserve a symposium of its own. Even with this limitation, the subject to be covered is so vast that many parts of it can be touched upon only very briefly. Attention is directed chiefly to disease states of particular topical interest. You will note that many of the disease mechanisms discussed are so similar in both children and adults that a sharp demarcation between the age groups would be artificial.

Haemolytic mechanisms are at work in childhood considerably more often than is usually recognized. Furthermore the application of the complete armamentarium of serological and chemical laboratory methods of to-day leave an ever-diminishing group of cases which are not clarified with respect to mechanism and severity of disease.

Sixten Sjölin[2] Clinical picture classification, and diagnosis

The clinical picture in haemolytic states varies greatly. On the whole there is a close relationship between the clinical picture and the degree of haemolysis, pronounced haemolysis giving pronounced clinical signs. On the other hand, the clinical picture is *fundamentally* independent of such factors as the cause of the haemolysis and the patient's age. The only striking and important exceptions to this rule are seen during the *first months of life* (**).

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In marked haemolysis the presenting signs are pallor lassitude restlessness, jaundice pains in the back and extremities, pyrexia, palpitations, and dyspnoea. The clinical picture and preliminary examination alone will usually suffice to give the diagnosis in such cases. There is always anaemia, and as a rule hyperbilirubinaemia, marked reticulocytosis, and anisocytosis. Normoblasts are often found in the peripheral blood. There is also commonly leukocytosis and a "shift to the left" Haemolysis may be so rapid that haemoglobinaemia and haemoglobinuria result The serum haptoglobin is practically always nil. There is always increased excretion of urobilinogen in urine and faeces. Splenomegaly is common, and enlargement of the liver may occur as well.

Slight hyperhaemolysis, however is most commonly characterized by the absence of physical signs. There is sometimes slight jaundice, with no anaemia and no complaints on the patient s part. In more chronic states, for example, some cases of congenital spherocytosis, the patient may remain symptom free for long periods. In such cases attention is often drawn to the illness only during acute exacerbations or through attacks of abdominal pain or symptoms of gall bladder disease The diagnosis in such cases may be very elusive, and may be entirely dependent on the physician powers of observation. Particular vigilance is called for when there is no anaemia. The degree of hyperhaemolysis is then so modest that the bone-marrow by increasing its production, is able to compensate for the loss due to haemolysis. Such cases of compensated haemolytic disease may be associated with hyperbilirubinaemia, at any rate at times, and the haptoglobin level may be low but slight reticulocytosis is commonly the only sign. The reticulocytosis may never be regarded as more than an indirect sign of hyperhaemolysis, however and t is never permissible to base the diagnosis of a haemolytic disorder solely on the finding of an increased reticulocyte count. It is in the case of these patients in particular who are relatively free from symptoms, that the clinician requires the help of the special laboratory to determine the red-cell survival and thus to arrive at the true diagnosis.

When the investigation of a case has proceeded so far that hyperhaemolysis has been demonstrated, there remains the important task of establishing exactly what type of haemolytic disorder the patient is suffering from. Even though our knowledge of the causes of the haemolytic states is still in complete, the clearest and most useful classification from the point of view of the clinician is that based on pathogenesis. In general these states may be divided into 3 groups (Table 1) The first includes those disorders in which the basic fault is a defect in the red cells. In the second group we have the cases in which normal red cells are produced and the haemolytic process is caused by some extracorpuscular factor Finally the third group includes cases in which it is known that haemolysis is due to a combination of intra- and extracorpuscular factors.

The chances of distinguishing these three groups are as a rule fairly good, but may be difficult if the patient has recently received a blood transfusion. If possible blood should not be administered until the diagnosis is clear

In this paper I am only going to discuss some of the most important of these haemolytic states and only those factors and laboratory tests that are not coming up later in this Symposium.

Intracorpuscular defects

Most f the haemolytic anaemias due to intracorpuscular factors are hereditary In these states a good deal of help is, of course to be had from the family history and investigation of the patient's relations, but it must be remembered that there are patients with hereditary haemolytic anaemia whose parents and siblings are healthy Hereditary haemolytic anaemia may produce symptoms at birth. In this connexion it must be stressed that the red cells of the newborn are macrocytic and contain foetal haemoglobin,

TABLE 1 *Classification of haemolytic disorders*

Intracorpuscular defects
- Hereditary spherocytosis
- Hereditary elliptocytosis
- Hereditary non-spherocytic haemolytic anaemias
- Congenital Heinz-body anaemia
- Congenital haemolytic anaemia associated with porphyria
- Sporadic congenital spherocytosis associated with congenital hypoplastic thrombocytopenia and malformations
- Infantile pyknocytosis?
- Thalassaemia
- Haemoglobinopathies
- Paroxysmal nocturnal haemoglobinuria

Extracorpuscular causes
- Haemolytic anaemias caused by isoagglutinins
  - Haemolytic disease of the newborn
  - Mismatched transfusions (anti A, anti B anti Rh, etc.)
- Idiopathic haemolytic anaemias
  - Antibodies demonstrable (auto-immune haemolytic anaemias)
  - No antibodies found
- Symptomatic haemolytic anaemias
  - Leukaemia, malignant lymphomas, osteopetrosis, disseminated lupus erythematosus, thrombotic thrombocytopenic purpura, cirrhosis of the liver
- Haemolytic anaemias caused by infections
  - Malaria, virus pneumonia, infectious mononucleosis
- Haemolytic anaemias caused by chemical agents
  - Phenylhydrazine naphthalene, aniline lead, sulphanilamide vitamin K, etc.

Combination of intra- and extracorpuscular factors
- Chemical agents primaquine naphthalene, sulphanilamide (vitamin K?)
- Vegetable agents: fava bean

and that their enzyme pattern is different from that of red cells of adults [97], and their osmotic resistance is higher [80]. The characteristic blood picture of several hereditary haemolytic syndromes is therefore often modified in the neonatal period and during early infancy [10, 27].

Hereditary spherocytosis is inherited as a Mendelian dominant and is most frequently recognized for the first time during childhood or early adulthood, but cases have been diagnosed at or within a few days of birth [56, 78]. The chief feature characterizing the red cells in this condition is microspherocytosis. And providing the technique is careful, the osmotic resistance can usually be shown to be decreased. The chances of demonstrating the anomaly of the red cells are improved if the cells are first incubated at 37°C for 24 hours [19]. The osmotic resistance is then usually found to be greatly lowered. The degree of spontaneous haemolysis is increased after incubation; and if glucose is added autohaemolysis is diminished by the same degree as in normal red cells [19]. Aplastic crises are seen in hereditary spherocytosis. These cases are characterized by a sudden cessation of bone marrow [illegible] with varying degrees of anaemia, [illegible]penia, and thrombocytopenia. From [illegible] rather extensive studies that have [illegible] carried out it now seems likely that [illegible] haemolysis in hereditary spherocytosis [illegible] due to a genetically determined [illegible] metabolism of the red cells [illegible].

Non-spherocytic haemolytic [illegible] seems to be inherited as a [illegible] nant. This disease which [illegible] has been described in [illegible] may resemble [illegible] tals [53]. In most [illegible] been published, [illegible] and anaemia were [illegible] fancy. There are [illegible]

the osmotic resistance is normal. Selwyn & Dacie have shown that there are at least two main types of non-spherocytic anaemias [76] Type I is characterized by a moderate degree of ovalocytosis; there is a tendency to abnormally increased osmotic resistance after incubation; the autohaemolysis rate is within the normal range or only slightly in creased, and the addition of glucose reduces the degree of autohaemolysis, but less so than with normal cells. Type II cases have rounded macrocytes; after incubation the osmotic resistance of the red cells decreases markedly; the rat of autohaemolysis is strikingly increased, and the addition of glucose increases rather than decreases autohaemolysis. Marked basophilic stippling is sometimes found and it is possible that these cases form a third group [19]. The essential defect in the red cells of hereditary non-spherocytic haemolytic anaemia seems to be biochemical and to differ in nature from that in hereditary spherocytosis [77 98].

Congenital haemolytic anaemia with marked Heinz-body formation is a rare disease, predominantly affecting premature infants and probably caused by a congenital defect of the red cells [4, 19]. As a rule the disorder is transient (although sometimes fatal), but some cases seem to persist int late child hood. This group includes only spontaneous cases, that is, those cases where no toxic agents such as aniline, sulphanilamide, or vitamin K can be incriminated.

I fantile pyknocytosi is a new" disease quit recently described by Tuffy Brown & Zuelzer [89]. They published the cases f 11 newborn infants, both premature and full-term, with acute severe haemolytic anaemia characterized by an increased number of pyknocytes or burr cells. These cells are distorted and contracted red cells with spiny projections, and seem to occur regularly in small numbers in infants up to the age of 2–3 months. The disease is associated with neonatal hyperbilirubinaemia. It is transient, and responds to transfusion therapy Genetic factors probably play a part in its development.

Thalassaemia minor is ften difficult to diagnose. It represents the heterozygous state of a partially dominant autosomal gene and is characterized by the presence of nu merous target cells, raised osmotic resistance, and increased concentration of haemoglobin A_2. In many cases small quantities of foetal haemoglobin can also be demonstrated The rate of destruction of red cells is usually nor mal. *Thalassaemia major or Cooley's anaemia*, represents the homozygous condition. The illness usually makes its first appearance during infancy The most typical features are marked haemolytic anaemia with pale, irregular sometimes fragmented red cells and target cells, increased osmotic resistance and a high concentration of foetal haemoglobin No abnormal haemoglobin is found. Thalassaemia is therefore not to be consid ered a true haemoglobinopathy It is not confined solely to persons of Mediterranean ancestry

Paroxysmal nocturnal haemoglobinuria is a disease of adults, and very few cases have been described in children [18]. There is never a family history of the condition, in spite of the disease being due to an intra corpuscular defect

Extracorpuscular causes

Haemolytic anaemias due to extracor puscular factors are usually classified as acquired haemolytic anaemias. A large number of these anaemias are due to antibodies. Our most important diagnostic aid in these cases is Coombs's direct test.

Haemolytic disease f the newborn is char acterized by the fact that the antibodies are transferred passively The haemolytic process is conseq ently transient, the degree of haemolysis subsiding parallel t the natu ral decay of the immune globulins.

Idiopathic haemolytic anaemias. Among the haemolytic anaemias there are always cases in which no aetiological factor can be discovered. These are classed as idiopathic haemolytic anaemias. The clinical picture varies widely but the signs are rarely as mild as those sometimes encountered in hereditary spherocytosis. In children the

onset is often abrupt. Cases with an acute onset, short duration, and spontaneous recovery have often been referred to as Lederer anaemia. In most cases Coombs's direct reaction is positive and warm antibodies can usually be demonstrated. In these cases the disorder is called auto-immune haemolytic anaemia. The fact that antibodies cannot be demonstrated in certain cases may be due t imperfections in the serological technique. Idiopathic haemolytic anaemias can be accompanied by thrombocytopenia. Aplastic crises are rare. Idiopathic haemolytic anaemias occur rarely during the first months of life, probably because the capacity for forming antibodies is then slight. The youngest patients described were ½, 5 and 5½ months old [18, 55].

Symptomatic haemolytic anaemia. Since reliable methods have become available for the measurement of the life-span of the red cells it has been found that the usually slight, but sometimes grave, anaemia that accompanies many prolonged, serious disease states is commonly at least in part, due to shortened red-cell survival. Sometimes cases of this type may show marked haemolytic signs. In several cases of symptomatic haemolytic anaemia Coombs direct test has been positive, and it is probable that the mechanism of haemolysis is similar t that in the auto-immune haemolytic disorders. A number of the states that may cause symptomatic haemolytic anaemias occur during childhood, for example leukaemia, thrombotic thrombocytopenic purpura, osteopetrosis, lupus erythematosus, and cirrhosis of the liver [1., 18, 82, 88, 92, 93].

Haemolytic anaemias due to virus fections are rare in childhood. Virus pneumonia and infectious mononucleosis seem to be the commonest causes, but cases have also been described following measles and herpes simplex [18, 35 67]. Antibodies have been incriminated, and in cases following virus pneumonia they have been f the cold" variety

Haemolytic anaemias caused by chemical agents. Among chemical agent that may induce haemolytic anaemia synthetic water soluble vitamin K is of special interest t the paediatrician [4 96, 97]. It is now cert in that excessive doses of vitamin K given to new-born premature infant may result in progressive haemolytic anaemia during the first weeks of life. It is uncertain, however whether this is true of all premature infants or only of those with sensitive cells (see below). The blood picture is characterized by large numbers f H inz inclusion bodies and numerous distorted and fragmented red cells. The disease is self-limiting, but death from kernicterus has been reported.

Combination of intra and extracorpuscular factors

Concerning the third main group, combined intra and extracorpuscular factors, I should first like to point out that they provide examples of a "new" mechanism of haemolysis. It has for long been known that certain persons, chiefly negroes develop haemolytic anaemia fter receiving the antimalarial drug primaquine and that others, mostly of Italian and Greek descent develop haemolytic anaemia after eating f va beans or after inhaling fava-bean pollen [9]. It has since been shown that the red cells of these persons are sensitive to the respective agents, exposure leading to haemolysis. Owing to an inherent biochemical bnormality these primaquine-sensitive cells form Heinz bodies more readily than non sensitive cells. It is highly probable that these findings will also prove significant in the elucidation of other haemolytic anaemias caused by chemical agents in sensitive persons. There is, for example much to suggest that some vitamin K naphthalene-, and sulphanilamide-induced haemolytic anaemias belong to this group [94].

The mportance of red-cell survival studies It is clear that with the aid of fairly simple laboratory test we are often ble to establish with certainty the nature of gi en patient haemolytic anaemia. On the other hand, it m st be stressed that advanced laboratory facilities are frequently required in order t arrive t the correct diagnosis. T

TABLE 2 *Summary of the investigation of a patient with suspected haemolytic anaemia* (Modified after DE GRUCHY 1958.)

History
- Age at onset of symptoms
- Race
- Jaundice
- Colour of urine and faeces
- Crises
- Cholelithiasis
- Family history
- Symptoms suggestive of disorders causing symptomatic acquired haemolytic anaemias
- Drugs

Physical examination
- Jaundice, splenomegaly hepatomegaly
- General development, height, facies, presence of congenital abnormalities, leg ulceration or pigmentation (hereditary haemolytic anaemias)
- Signs of disorders causing symptomatic acquired haemolytic anaemia, especially lymph-node enlargement and purpura

Special investigations

A. *Essential investigations for all cases*

Full blood examination, with special reference to:
- Morphology of red cells in a well-made and well-stained blood film (note especially spherocytosis, auto-agglutination, fragmentation, inclusion bodies)
- Reticulocyte count
- Serum bilirubin estimation
- Osmotic resistance
- Direct Coombs test
- Examination of urine for urobilinogen, haemoglobin, haemosiderin

B *Further investigations required in some cases*
- Estimation of faecal urobilinogen
- Measurement of red-cell life span with Cr^{51} (or DFP^{32}) combined with *surface counting over* liver and spleen
- Measurement of red-cell destruction with carbon monoxide
- Autohaemolysis after incubation, with and without added glucose (hereditary spherocytic and non-spherocytic haemolytic anaemias)
- Osmotic resistance after incubation (hereditary spherocytosis)
- Examination of plasma for haemoglobin and methaemalbumin
- Estimation of haptoglobin concentration
- X-ray of skull, hands, and long bones (hereditary haemolytic anaemias)
- Sickle-cell test (sickle-cell anaemias)
- Tests for abnormal haemoglobin types (hereditary haemolytic anaemias)
  - ( ) Paper starch block, or agar gel electrophoresis
  - (*b*) Chromatography
  - ( ) Alkali denaturation technique
- Investigation of relatives (hereditary haemolytic anaemias)
- Examination of cells for methaemoglobin and sulphaemoglobin (chemical haemolytic anaemia)
- Heinz-body preparations (chemical haemolytic anaemia)
- Serum cold agglutinins and warm agglutinins (acquired haemolytic anaemias)
- Investigations t demonstrat aetiology in symptomatic acquired haemolytic anaemia, especially LE-cell test and lymph node biopsy
- Wassermann test (paroxysmal cold haemoglobinuria; N.B. false positives in acquired haemolytic anaemias)
- Donath–Landsteiner test (paroxysmal cold haemoglobinuria)
- Ham acidified-serum test (paroxysmal nocturnal haemoglobinuria)

will be further illustrated at a later stage of the Symposium. In the present connexion I wish only to emphasize the value of repeated red-cell survival studies, especially in cases in which there is doubt concerning the main group to which the case should be assigned. I think this is best illustrated by an example. In a case of osteopetrosis that we examined we were able to demonstrate with the aid of radioactive chromium that the red cells of the patient had a reduced survival time following autotransfusion [32]. In other words, haemolytic anaemia was present. Chromium tagged red cells from a healthy donor behaved in the same manner when injected into the patient. These investigations thus established the fact that an extracorpuscular factor was at work, but it was not until the patient's chromium-tagged red cells had proved to survive normally in a healthy recipient that it became clear that hyperhaemolysis was due exclusively to an extracorpuscular factor. Similarly it is possible to demonstrate intracorpuscular defects, and even combined intra- and extracorpuscular factors. And survival studies on red cells of varying genotype are of great value in investigating the part played by different antibodies in causing haemolysis in individual cases [45].

When a haemolytic anaemia is suspected, a careful clinical and laboratory investigation is always needed in order to establish the correct diagnosis. In analysing such cases the procedures listed in Table 2 may serve as a guide.

Claes Högman[1] Serological aspects of pathogenesis and diagnosis

It is now generally accepted that acquired haemolytic anaemia (AHA) can be due to immunological mechanisms. Antibodies, either produced by another individual (isoantibodies) or by the patient himself (autoantibodies) react with antigens of the patient's erythrocytes, resulting in shortening of the life-span of the cells. Haemolytic disease of the newborn, as a rule due to passively transferred Rh or ABO antibodies, is the commonest immune haemolytic anaemia of childhood in our part of the world. This disorder will not be discussed at this Symposium, however.

In the other type of AHA, that due to autoantibodies, serological methods such as the antiglobulin reaction (Coombs's test) are positive, indicating the presence of antibody-like globulins on the patient's erythrocytes. It is not known, however, whether the globulins coating the red cells are true autoantibodies, or whether they are antibody-like pathological proteins. From a clinical point of view they may be regarded as true antibodies. They can be classified according to the temperature of their optimum reaction *in vitro* [18]. The warm antibody has its optimum at 37°C, whereas the cold reacts best at temperatures just above zero, with some activity up to 25°–32°C but seldom at 37°C. Certain other properties of the antibodies are correlated to the optimum temperature and are summarized in Table 3 (cited from Dacie [20]).

The anaemia caused by antibodies of the cold type is the rarest, and seems chiefly to affect patients above 40 years of age [23]. The warm type anaemia may appear equally frequently in children as in adults. The antibodies of the latter type are incomplete, that is, they are detectable by the antiglobulin technique but do not cause agglutination of red cells suspended in saline medium. The incomplete antibodies may, however, agglutinate red cells suspended in colloid rich medium such as albumin, gum acacia, dextran, or polyvinylpyrrolidone (PVP). Sometimes the most sensitive reaction is obtained if the red cells are treated with certain proteolytic enzymes such as trypsin, papain, or ficin [60]. Red cells coated with incomplete autoantibodies are usually not haemolysed in the presence of complement, as is often the case with the cold type antibodies [18, 19]. An exception from this rule is described by Dausset & Colombani [**]. In a series of 1?8 cases of AHA, four patients

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TABLE 3 *Characteristics of the auto-antibodies of acquired haemolytic anaemia* (After DACIE 1959)

	"Warm" type of antibody	"Cold" type of antibody
Nature		
Electrophoretic mobility	γ-Globulin	γ-Globulins (? β-Globulins)
Sedimentation constant	S_{20} 7	S_{20w} 16-19
Class of antibody		
Complete (agglutinating) or incomplete (non-agglutinating)	Usually incomplete	Complete
Haemolytic potentiality	Fails to haemolyse normal or trypsinized red cells or PNH red cells	Haemolyses normal red cells (sometimes only at pH 6.5-7.5), trypsinized red cells and PNH red cells
Antiglobulin reaction		
Presence of prozone	Frequent	No prozone
Effect of pH of patient serum	Little effect	Agglutination usually increased at pH 6.5-7.5
Effect of heating patient serum to 56° C	Little effect	Complete inhibition of agglutination
Effect of adding γ-globulin to anti-globulin serum	Inhibition by small amounts of γ-globulin	No inhibition by small amounts of γ-globulin
Thermal range of antibody	Maximal at approximately 37° C	Not active at 37° C; just active at 28-32° C; activity greatly increased by cooling *below* 28° C
Specificity of antibody	"Non-specific" usually; anti-Rh plus "non-specific" occasionally; anti-Rh alone or other specificities, rare	"Non-specific" Anti-I

had antibodies of the warm type which, against the rule, acted as haemolysins. Three of these cases were children, 8 months to 4 years of age, and they all showed a similar clinical picture with pyrexia, haemoglobinuria, jaundice and enlargement of the liver and spleen. The illness appeared after an upper respiratory infection, and rapid recovery occurred after transfusion and corticosteroid therapy.

The auto-antibodies may be found both in serum and on the patient's red cells or on the red cells only. They can be removed from the erythrocytes by elution and subjected to analysis [18, 51]. The cold auto-antibodies always appear non-specific in routine testing, i.e. they react with all samples of red cells independent of their blood groups. In contrast, the warm antibodies sometimes show a distinct serological specificity [49, 91], often within the Rh-system. Hollander & Batschelet [43] pointed out that the incidence of the different Rh-specific auto-antibodies is closely correlated to the incidence of the Rh-antigens in the population. Consequently, as the e-antigen is present in about 98% of all individuals, the anti-e would be expected to be one of the commonest specific auto-antibodies, and this, in fact, is the case. Dacie [20] suggests that the apparently non-specific antibodies (which are the commonest of the auto-antibodies) may also turn out to be specific. They could, in theory, be directed against a public antigen common to the vast majority of human individuals.[1]

[1] Support for this hypothesis has been given by Wiener, Unger, Cohen and Feldman (Ann.

In certain cases this specified diagnosis of the antibodies may not only be of theoretical interest but also of clinical value. It has been demonstrated [45, 61] in cases of AHA due to auto-anti-e that the patient's own red cells, which contained the e-antigen, were destroyed much more rapidly than transfused red cells lacking the e-antigen.

The clinical findings may indicate AHA, even though the serological reactions are all negative. The question of whether the disease is due to a non-immunological mechanism in these cases or whether the methods available are inadequate cannot be answered at present. It is to be remembered, however, that the reagent used in the antiglobulin test, which is considered to be the most reliable method available, is produced by animals, usually rabbits, and that the capacity of individual rabbits to form these antibodies varies greatly. The antiglobulin sera must be carefully selected, absorbed and tested, and should be used in at least two different dilutions [25, 60]. Furthermore, it is possible that in specific cases the patient's auto-antibodies may consist of pathological globulins not normally present and that normal human serum is therefore not capable of stimulating the animals to produce the relevant anti human globulins. By immunizing rabbits with the serum of a patient showing clinical signs of haemolytic anaemia, Evans & Weiser [28] obtained an antiglobulin serum which gave a positive antiglobulin reaction with the red cells of the patient in question although other antiglobulin sera failed.

Addition of suitable amounts of human gamma-globulin to antiglobulin sera inhibits the anti-gammaglobulin, but not anti-bodies against other globulins. Sera partially neutralized in this way react with red cells sensitized by certain types of human antibodies. This is true both with iso- and auto-antibodies [60]. Human auto-antibodies can thus be divided into a gamma-globulin and a non-gamma-globulin type. Recently it has been demonstrated [73] that the 'non gamma-globulin' reaction is due to rabbit anti-human globulin which combines not with the antibody itself but with human complement participating in the reaction.

The fixation of antibodies on the surface of erythrocytes apparently causes damage to the cells, reducing their life-span. The survival time of red cells has been studied by differential agglutination (Ashby technique) and by tagging red cells with radioactive isotopes, in recent years especially Cr^{51} [60, 61]. Both quantitative and qualitative factors influence the rate at which the antibodies bring about destruction of the cells. Rh-positive red cells coated with large amounts of Rh (D) antibodies survive for a far shorter time than cells coated with a smaller amount of antibodies [48]. In the acute phase of haemolytic anaemia antibodies are often found in the serum as well as on the surface of the red cells, whereas no free antibodies may be found during remissions [18]. This indicates that excess antibody is formed in the acute phase. The presence of free antibodies in the plasma has been found to indicate a poor prognosis [22].

In some cases the red cells can be shown to undergo intravascular lysis, apparently by the action of serum complement; in other cases they are destroyed with the co-operation of such organs as the liver and the spleen [44, 45, 48, 49, 50, 63]. In the latter event little or no free haemoglobin is to be found in the plasma. By measuring the radioactivity over different areas of the body it is possible to get a rough idea of the accumulation in different organs. This in turn indicates that the organ in question is involved in the process of red-cell sequestration. Erythrocytes coated with incomplete antibodies are generally trapped in the spleen, whereas complete antibodies cause sequestra-

Intern. Med. *11*, 221, 1956), who showed that 5 out of 22000 blood reacted only very weakly with potent cold agglutinins. They concluded that an antigen called I was lacking in the red cells of these 5 individuals. This antigen is poorly developed at birth and increases in strength continuously during the 2nd to 18th month of life (Marsh, Brit. J. Haematol. *7*, 200, 1961). The finding of Wiener *et al.* that the cold agglutinins show an anti-I specificity has been confirmed by Jenkins, Marsh, Noades, Tippett, Sanger and Race (Vox Sang. *5*, 97, 1960).

TABLE 4

Subcellular parts	Main function	Presence in the mature erythrocyte
Nucleus	Source of genetic information (from DNA)	–
Mitochondria	Production of energy (ATP) through oxidation	–
Microsomes	Synthesis of proteins (enzymes)	–
Supernatant fraction	Glycolysis to (pyruvic acid and) lactic acid	+
	Purine and pyrimidine metabolism	(+)

tration in the liver [44, 48 48, 50]. The site of destruction of the erythrocytes is also influenced by quantitative factors; the same serum may in small doses, cause red-cell sequestration in the spleen, in bigger doses also in the liver [50]. Increased destruction of red cells probably due to an immunological mechanism is sometimes observed, although none of the serological reactions is positive [2, 81 85].

As previously mentioned different co-factors are often necessary if an antigen-antibody reaction is to be apparent *in vitro*. This may well also be the case *in vivo*. For example deficiency of complement may lower the effect of complement fixing antibodies. The function of agglutinating factors present in pathological [28] or normal [43] sera is unknown. It is possible that such factors may increase the damaging effect of sensitization. Jandl *et al.* [48] found a higher rate of destruction of red cells from a patient with acquired haemolytic anaemia when the cells were injected into a patient with multiple myeloma than when they were injected into a healthy individual. The authors concluded that the hyperglobulinaemia was of importance. The occurrence of the agglutination *in vitro* of red cells coated with incomplete antibodies which takes place with the aid of certain large molecules would thus perhaps have a parallel *in vivo*.

Carl Henric de Verdier[1] Biochemical aspects of pathogenesis and diagnosis

The evolution of the erythroblast to a mature red blood corpuscle is an example of development to a highly specialized cell. The morphological characteristics are the disappearance of nucleus, mitochondria, and microsomes (Table 4). These different subcellular particles have different biochemical functions which will disappear at the same time, and this means that the mature erythrocyte will have a very restricted metabolic pattern. In an ordinary cell, most high-energy compounds such as adenosine triphosphate (ATP) are formed by oxidative phosphorylation when substrates such as pyruvate are degraded through Krebs citric-acid cycle in the mitochondria, and they are only to a smaller extent formed through glycolysis. In the erythrocyte ATP is formed only by glycolysis. When the haemoglobin synthesis is complete the microsomes will disappear and the cell can no longer synthesize proteins, which means that it cannot replace consumed enzymes. This is important to bear in mind when discussing the pathogenesis of haemolytic anaemia.

There are several different pathways of intracellular metabolism, related to the haemolytic disorders and glycolysis will first be discussed. Breakdown of glucose follows two pathways. Both of these have several names, but they will here be called the Embden–Meyerhof pathway and the pentose phosphate shunt (Fig. 1). Normally about 10% of the glucose is metabolized through the pentose phosphate shunt [63]. A derivative of vitamin B_1, thiamine diphosphate is involved in the catabolism along this shunt [94]. In some of the steps an oxidation or

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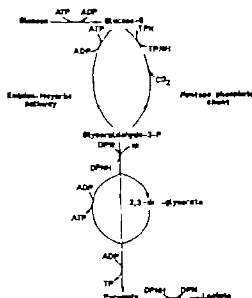


Fig 1. A simplified scheme of glycolysis in the erythrocyte. Abbreviations: ATP = adenosine triphosphate; ADP = adenosine diphosphate; TPN and TPNH = triphosphopyridine nucleotide in oxidized and reduced form. DPN and DPNH = diphosphopyridine nucleotide in oxidized and reduced form; IP = inorganic phosphate; P = phosphate.

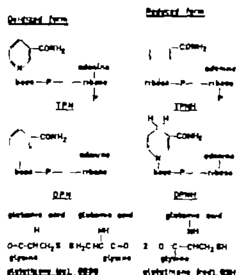


Fig 2. Main structure of TPN, DPN, and glutathione in reduced and oxidized form.

reduction takes place and these steps are catalysed by enzymes to which co-factors, called pyridine nucleotides, are coupled. There are two kinds of pyridine nucleotides, abbreviated DPN and TPN. Their main structure is shown in Fig 2. If they oxidize a substrate, they become reduced t DPNH and TPNH respectively TPN is the co-factor in the pentose phosphat shunt whereas DPN systems are only t work in the Embden-Meyerhof pathway In this pathway the DPNH first formed is con sumed in the final transfer to lactat so there is no net synthesis in the completed glycolysis reaction.

Work done by Altman and Frankerd and their associates [5, 6 69] has shown an b- normality probably deficiency of single enzyme in the glycolytic chain, in hereditary spherocytosis. Labelled inorganic phosphat enters the cells and is metabolized more slowly there than in normal red cells. Addition of fluoride exaggerates the difference. This finding and certain other evidence would suggest that the deficient enzyme could be phosphofructokinase or enolase

Some few investigations have also been made t clarify the defect in congenital non spherocytic haemolytic anemias. Probably here too the explanation may be inborn errors of met bolism, but the sites of these errors are probably different from the set in hereditary spherocytosis.

In the red cells the TPN is mainly present in the reduced form, TPNH. This means that the content f TPN will normally determine the rate of degradation along the pentose phosphat shunt The pyridine nucleotide systems are generally in equilibrium with other reduction-oxidation systems in the erythrocyte (Fig), namely the gl system, which is present in high

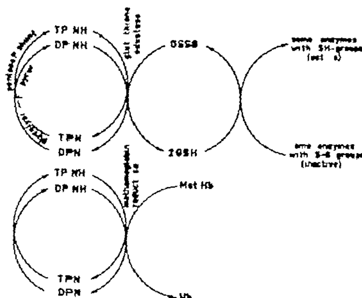


Fig. 3 Interaction of some oxidation-reduction systems in the erythrocyte.

the haemoglobin-methaemoglobin system. Some vital enzymes [71–75] are active only in a reduced state and it is important for them that the equilibrium of glutathione is shifted towards the reduced form (Fig. 3). If oxidized glutathione or methaemoglobin is present it will oxidize TPNH to TPN and thus accelerate the reactions of the pentose phosphate shunt.

Investigations have shown that about 7% of an American Negro population have deficient activity of the first enzyme in the pentose phosphate shunt. The enzyme is called glucose-6-phosphate dehydrogenase and its activity is reduced to about one-quarter to one-tenth of the normal value [e.g. 37]. Similar enzyme deficiency has also been found to a lesser extent in Caucasian populations. The subjects show no clinical signs except after taking certain drugs such as primaquine, sulphanilamide or naphthalene or after eating fava beans, when they exhibit a haemolytic disorder, often with methaemoglobinaemia and Heinz bodies [6]. These substances are thought to inhibit the enzyme glucose-6-phosphate dehydrogenase [23], thus blocking the pentose phosphate shunt and the TPNH production. The enzyme defect can be discovered either by direct enzyme determination or by estimation of the ability of the cells to keep glutathione in a reduced state after treatment with acetyl phenyl-hydrazine, the so-called glutathione stability test. An Iranian family has also been described in which the members had no measurable amounts of glucose-6-phosphate dehydrogenase in their red blood corpuscles [57]. These patients suffered from spontaneous episodes of haemolytic activity and increased osmotic fragility.

Vitamin K catalyses the oxidation of TPNH to TPN, thus accelerating the pentose phosphate shunt. In this case the increased breakdown of glucose along the shunt is not accompanied by an increased net production of TPNH. The enzymes of the shunt are present in the red blood corpuscles in higher concentrations in the newborn infant than they are later. Both the increase in TPN and the elevated concentrations of the enzymes of the shunt contribute to increase the catabolism of glucose along the shunt. Especially in the newborn infant with its physiological hypoglycaemia, this may lead to deficiency of glucose in some parts of the circulation. Both factors, the

lack of glucose and the presence of vitamin K, thus tend to diminish the content of TPNH in the red cells, which may be fatal for the cells and cause haemolysis [97].

In paroxysmal nocturnal haemoglobinuria (PNH) the haemolytic disorder is probably not due to an error in glycolysis. The findings of a low content of the enzyme acetyl cholinesterase [7] and a low content of the lipid lecithin [70] would suggest an error in lipid metabolism. The membrane of the erythrocyte is said to be a mosaic of lipids and proteins and a fault in the membrane would therefore be the most likely explanation for this haemolytic disorder.

Few investigations have been made on the metabolism of the red cells coated with antibodies [1, 38, 86], and more work needs to be done before it is possible to interpret the results obtained.

It is likely in genetic disorders that some degree of the enzyme deficiency exists in all cells of the organism, but cells with specialized metabolism would probably suffer more from a metabolic block owing to the limited chances of bypassing the block or of producing essential metabolites via other routes. This is the case in erythrocytes, where ATP and reduced substances such as TPNH can only be formed by the breakdown of glucose to lactate or pyruvate. In the erythrocyte the ATP is essential for the transport of ions [24, 68] and thus for maintaining the right internal environment, and the TPNH is used for keeping some enzymes in their reduced, active state. This may explain why genetic disorders and other conditions affecting glycolysis produce the clinical sign of haemolysis.

Stig Sjölin[1] Abnormal haemoglobins: pathogenesis, and diagnosis

In 1949 Pauling, Itano, Singer & Wells demonstrated the presence of an abnormal

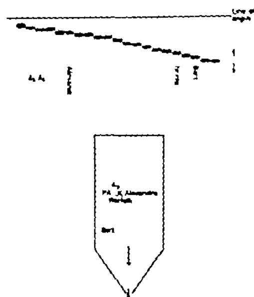


Fig. 4. Upper part: mobilities on paper electrophoresis at pH 8.6 of carboxyhaemoglobins. Lower part: sequence of separation of haemoglobins in an Amberlite IRC-50 ion-exchange column at pH 6.2 (the diagrams are taken from DACIE, *The Haemolytic Anaemias*, Part 1, pp. 292, 293, 1960).

haemoglobin in sickle-cell anaemia [65]. This haemoglobin was called Hb-S. In 1950 Hb-C was described, in 1951 Hb-D and in 1954 Hb-E. Between 1954 and 1958 nine new haemoglobins were found, and lettered from H to Q. Since then at least seven probably new haemoglobins have been reported.

A child who has received the normal Hb-A from one of his parents and an abnormal haemoglobin from the other represents the heterozygous state. This condition is referred to as a trait. As a rule the heterozygous state is clinically harmless.

When both parents transmit the same abnormal haemoglobin to the child, the condition represents the homozygous state. The term disease is used to describe a condition produced by one or two haemoglobin variants in the absence of Hb-A. Several of these haemoglobin diseases are characterized by

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clinical and haematological signs, sometimes severe In some of them increased red-cell destruction is a typical finding In Hb-S disease or sickle-cell anaemia, the life-span of the red cells is considerably diminished. The combinations of Hb-S with thalassaemia, Hb-C, or Hb-D lead to clinically significant excess haemolysis, although this is less severe than in Hb-S disease. In Hb-C disease the life-span of the red cells is moderately reduced. In Hb-D and Hb-E disease and in Hb-E/thalassaemia the red-cell survival is only slightly shortened.

The most important diagnostic methods used to detect the haemoglobinopathies are paper starch block, or agar gel electrophoresis, chromatographic separation, resistance to alkali, and solubility measurements. The relative mobilities of the different haemoglobins at pH 8.6 on paper electrophoresis and on resin-column chromatography are seen in Fig. 4. Most, but not all abnormal haemoglobins can be separated by electrophoresis. In several cases, however other methods are needed. Thus Hb-H and Hb-I, which show the same relative mobility on paper electrophoresis, can be differentiated by chromatography Hb-S and Hb-D on the other hand show the same relative mobilities on paper electrophoresis and on chromatography and can be differentiated only by their different solubilities. Reduced Hb-S has a very low solubility while Hb-D is readily soluble

As early as in 1950 Pauling and his collaborators were able to show that the difference between Hb-A and Hb-S lay in the globin part of the molecule [68]. During recent years Ingram has been able to explain the nature of the differences between some haemoglobins [48]. The only difference found between Hb-A and Hb-S was that one amino acid out of 300 forming the two polypeptide chains of a half molecule was substituted. One of the normal glutamic acid residues was replaced by valine In Hb-C the same glutamic acid was also replaced but this time by the amino acid lysine And similar abnormalities have been demonstrated in some other haemoglobins. It is interesting to note that such very minute molecular changes can result in marked physico-chemical differences and sometimes in severe disease.

It seems now also clear that these chemical alterations in the haemoglobin molecule are caused by mutation of one of two haemoglobin genes. The chemical nature of each of these genes is thought to determine the amino acid sequence of the corresponding polypeptide chain. It is now a generally accepted view that the anaemia of the haemoglobinopathies can arise in one of two ways. In Hb-S disease the basic cause seems to be an increased rate of haemolysis linked directly to the presence of sickled cells in the peripheral blood, and thus to the molecular abnormality of the haemoglobin. In the other haemoglobinopathies, which are associated with a diminished life-span of the red cells, there is no evidence of a direct connexion between the molecular abnormality and the diminished red-cell survival. Undoubtedly however an intracorpuscular defect is to blame. In several haemoglobinopathies a slight retardation in the maximum rate of haemoglobin synthesis contributes to the anaemia.

Finally I would like to stress that we may expect to find abnormal haemoglobins in patients from all over the world, not only among Negroes and in the Far East. Thus in Norfolk three members of a "pure English family were found to be heterozygous carriers of a new haemoglobin not yet completely characterized [3]. At our department of paediatrics we have diagnosed Hb-H disease in two brothers [41]. And Wallenius & Höglund have since found haemoglobin H in an adult from Uppsala, who is not related to the two boys [90]. In Umeå Bergström & Jacobsson [8] have described several cases of an inherited non-haemolytic anaemia associated with an abnormal haemoglobin, as yet not identified. From Gothenburg, Hansen and collaborators [40] have reported a family with hereditary cyanosis probably caused by the presence of an abnormal haemoglobin very similar to Hb-M of the Boston type

Lars Garby[1]: Determination of red-cell destruction

Previous speakers have discussed several different processes and disease states in the human organism that may prove deleterious to the red cells. These cells may suffer in that their ability to carry oxygen and carbon dioxide becomes impaired, but above all they may suffer in such a way that they die and disappear from the circulation before their normal life-span is ended.

No matter what the mechanism of the premature destruction of red cells may be, their mean life-span will be shortened. The effect of this upon the number of circulating cells will depend on how the organism can adapt itself to the new situation. The relation between the characteristics of practical interest is

$$D = \frac{N}{T}$$

where D is the rate of red-cell destruction, N is the number of circulating red cells, and T is their mean life-span. If the mean cell life-span is shortened, the number of circulating red cells would diminish unless the bone marrow could increase its production of red cells. In cases of chronic haemolytic anaemia the production has been shown to be increased by a factor of 6–7 and in anaemias of shorter duration by a factor of 2–3 [16, 31]. When the maximum production capacity of the bone marrow has been reached, the degree of anaemia is a direct measure of the severity of the haemolytic process.

In what follows, I shall try to comment shortly on some methods in use for assessing the degree of red-cell destruction.

First, a few words about the measurement of the behaviour of labelled red cells. There are two different types of labelling of the patients' own cells. In the first procedure red cells are labelled during their formation, that is, a uniform age group of cells is labelled, for instance with the help of radioactive iron. An example of the use of this method is illustrated by a case of chronic refractory anaemia published by Garby, Sjölin & Vahlquist [30]. This patient had a chronic refractory hypochromic anaemia with a normal chromium-survival curve, indicating that the great majority of the circulating red cells had a normal life-span. Since there was an anaemia with about 7 g% of peripheral haemoglobin, the rate of production of normal cells was reduced to about half its normal value. However, the facts that the bone marrow was hyperplastic and the rate of disappearance of the plasma iron was greatly increased indicated that the production of haemoglobin was normal or raised. The incorporation of iron into circulating red cells was slow and reached a plateau at a value far below the normal range. These facts are probably best interpreted as reflecting the production of cells with very short survival, probably due to some intrinsic defect.

The second, more commonly used procedure of tracing the fate of labelled red cells involves labelling of peripheral red cells, that is, the circulating cell population is uniformly labelled with respect to the age of the cells. The mean life-span is obtained by drawing the tangent to the disappearance curve at zero time [59]. This tangent cuts the time axis at the mean cell life-span. In practice red cells are labelled with radioactive chromium or with di-iso-propyl fluoro-phosphonat (DFP) which is labelled in the phosphorus atom. In the case of the chromium method, there is a complication in that the chromium disappears from the circulation not only because of red-cell death but also ... elution of the chromium from the red cells. The chromium disappearance graph in ... normal individual is therefore curved ... straight line. As ... measure of the ... disappearance, that is, of the ... haemolysis, the time taken to reach half the initial value is usually given ... individuals, this time is betw... days [29], corresponding ... span of between 80 and 1... means that the red-cell ... about doubled if it is to be ... chromium method. An ...

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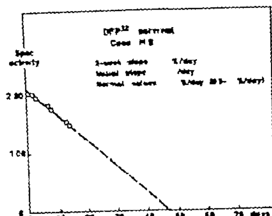


Fig 5. Red-cell survival measured with DFP[32] in a case of pancytopenia. Mean cell lif span about 47 days.

about 4 weeks is necessary in order to a hieve this precision. D iso-propyl fluorophosphonat (DFP) is an organic phosphorus compound that binds irreversibly to certain proteins in the red cell [14]. It is not eluted n vivo and gives straight lines in normal individuals with a red-cell life-span of 120 days. In our laboratory this method has been used with the same precision as the hromium method with only about 3 weeks of observation. An example is shown in Fig. 5. This patient had had a pancytopenia for about 6 months, and the disappearance curve shows a relative rate of destruction of about 3 times the normal The fact that there was anaemia with a haematocrit reading of about 20 implies that the absolut destruction rate was only about 1 5 t mes the normal, and that the capacity f the bone marrow to produce red cells was diminished.

It is a well established fact that the haem part of the haemoglobin molecule is not reutilized for new synthesis of haemoglobin. It is excreted in the form of urobilin and urobilinogen, and perhaps also in the form of dipyrrols [3., 33 79]. It might therefore be expected that the excretion rat f such products could be used as an index f red cell destruction. However determinations of the urobilinogen excretion in normal subjects generally give values that are lower than the expected ones, and there are several different sources of error complicating the interpretation. The excretion should preferably be related to the total haemoglobin mass. Values well above the upper limit of, say 25 mg per 100 g of circulating haemoglobin can be taken as good evidence of increased red-cell destruction.

Another catabolic product of haem has received attention during recent years. It appears that normal man excretes small amounts of carbon monoxide through the lungs [83], and that this compound is formed when one of the methene bridges of haem is broken. If the excretion rate of carbon monoxide is increased the alveolar carbon-monoxide concentration ought to rise In fact, a rather good correlation has been demonstrated between the alveolar carbon monoxide concentration and the degree of haemolysis as measured by the chromium method [26]. If this measurement can be put on a saf physiological basis, it will have definite advantages over methods that involve labelled cells, since it can be repeated, and only requires an observation time of a couple of hours.

It has been shown that under normal conditions only a very small proportion of red cells die intravascularly In some haemolytic disorders there are reasons for believing that the number of red cells which die intravascularly is much larger When free haemoglobin enters the circulation, it will form a complex with normally occurring plasma proteins called haptoglobins. The haemoglobin–haptoglobin complex is rapidly cleared from the plasma, presumably by way of the reticuloendothelial system in the liver spleen, and bone marrow The haptoglobin concentration in the plasma is lower the greater the rate of intravascular destruction. When the apparent half lif is less than 17 days, using the Cr[51] method the haptoglobin concentration is virtually nil [64]. The large variation in haptoglobin levels between normal individuals, that is between some 30 and 180 mg — limits the usefulness f the method.

An indication of the degree of haemolysis can also be obtained from measurements of

the red-cell production if the individual is in a steady state, that is, if the haematocrit readings are reasonably constant. The number of circulating reticulocytes has also been used for this purpose. Provided that the life-span of the circulating reticulocyte is constant, the number of circulating reticulocytes should be a good measure of the rate of red-cell production. Little is known about the life-span of the circulating reticulocyte under pathological conditions. Furthermore the error of reticulocyte counts is usually rather large, so that in practice reticulocyte counts of at least 3–4 times the normal mean value must be required to establish with certainty that the rate of production is increased. Repeated counts by technicians not knowing the previous values should help the situation considerably. Reticulocyte counts should preferably be given in absolute numbers and not relative to the number of circulating red cells, since relative numbers may be misleading in cases of anaemia.

Andreas Killander: Therapy

The therapeutic approach to the haemolytic anaemias is mostly empirical and is only to a small extent based on results obtained with the specialized and refined diagnostic methods that are available. Regardless of the aetiology of the haemolytic disorder, one or more of the three main methods of treatment has to be employed. These methods comprise blood transfusions, cortisone or ACTH treatment, and splenectomy.

Blood transfusions may be life saving in patients with rapid blood destruction and severe anaemia. Sometimes the presence of autoantibodies makes the matching of blood difficult. The patient's blood should therefore be genotyped before the first transfusion, and the specificity of his antibodies determined if possible. Except for emergencies, blood transfusions should be restricted to patients for whom no other treatment is possible. The aim should be not to achieve a haemoglobin level as low as possible to normal, but to keep the patient at the lowest haemoglobin concentration that he can tolerate without suffering. This level varies with different patients and different ages. A haemoglobin level of about 7 seems to be sufficient in most children. Vigorous transfusion therapy depresses the activity of the bone marrow and increases the risk of subsequent transfusion reactions due to isoimmunization and in the long run also of haemosiderosis and haemochromatosis. For the same reason iron therapy should be refrained from unless there is evidence of iron deficiency. This is very rarely the case in haemolytic anaemia.

In newborn infants with congenital haemolytic anaemia the accumulation of bilirubin sometimes becomes so excessive as to require exchange transfusion. The indication for this procedure depends much more on the degree of hyperbilirubinaemia than on the haemoglobin level.

The beneficial effect of cortisone and its derivatives and of ACTH in a number of blood diseases is now well recognized, but the mode of action of these hormones has not yet been clarified. It is beyond the scope of this paper to try to evaluate the evidence for and against the different explanations which have been put forward. Let it suffice to say that there is some evidence that cortisone may suppress the production of antibodies. No effect has been demonstrated on the circulating antibodies. The initial doses should be high, and equivalent to 300–400 mg of cortisone in the adult. This corresponds to 60–80 mg of prednisone which is at present the most widely used preparation. Treatment should be continued until the rate of haemolysis decreases, and then gradually reduced to the smallest amount necessary to keep the illness under control. If there is no effect within two weeks, it is improbable that the drug will work later on. It is uncertain whether ACTH may be helpful when cortisone fails.

The spleen is generally considered to be important in two ways. It is one of the sources of antibodies and it removes damaged,

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spherocytic and sensitized red cells from the circulation. The latter function is by far the most important in haemolytic anaemia. Following splenectomy in patients with autoimmune haemolytic anaemia little or no changes in the serological findings take place as a rule. In almost all patients splenectomy results in some lowering of the rate of haemolysis. If however the rate of haemolysis before the operation greatly exceeds the patient's powers of compensation, a small improvement in the red-cell life-span following splenectomy may be of little clinical benefit. On the other hand, if equilibrium between destruction and formation can be achieved or nearly achieved, removal of the spleen may lead to significant improvement. It is difficult or impossible to predict the result of splenectomy in such cases. Surface counting of radioactive chromium over the spleen and liver may provide a useful guide [*0]. High radioactivity over the spleen compared with the liver indicates preferential destruction of the red cells in the spleen. This finding is usually associated with a favourable result of a subsequent splenectomy. A low relative splenic radioactivity has been found in many patients who subsequently failed to respond to splenectomy. However there is not as yet enough information available to warrant the conclusion that all patients with a low relative accumulation of radioactivity in the spleen fail to respond to splenectomy.

In haemolytic anaemia secondary to other disease splenectomy may be of value in selected cases. The risk of post-operative complications in this group is high, and the effect is often of doubtful value.

This leads us to consider other possible contraindications to splenectomy. It is known that agenesis of the spleen may be associated with increased susceptibility to infection [47]. In 195 King & Schumacker [62] reported the occurrence of fatal infections in all of five infants who had undergone splenectomy at less than 6 months of age. Since then reports from other clinics have lent support to their observation [44, 63]. Contradictory results however have also been reported [54, 66]. In a recent well-documented paper Robinson & Sturgeon [7] report their experience of 110 splenectomized children followed up for a median period of 6 years. They divided their series into two groups. The first comprised 63 patients considered not to be predisposed to infections. One infant 4 months old at the time of splenectomy developed meningitis from which he recovered. The second group included patients with a variety of underlying disorders which were thought to predispose to infection. In this group of 47 patients, 12 developed severe infections after operation. From these results, and from findings recently reported by other workers, it is evident that splenectomy carries a very low risk of post-operative infection when carried out over the age of 6 months for the generally accepted indications of traumatic rupture of the spleen, hereditary spherocytosis, and idiopathic thrombocytopenic purpura. Since the possibility cannot be entirely excluded that splenectomy in early life may carry a small hazard, it seems reasonable to postpone the operation until at least beyond the limit of 6 or possibly 12 months.

In 1956 Dameshek published the observation of exacerbation of lupus erythematosus following splenectomy for idiopathic thrombocytopenic purpura and auto-immune haemolytic anaemia [21]. This report evoked a most controversial discussion, which is still going on. It appears that most workers in this field do not share Dameshek's view in this respect. And in fact it has been reported that haemolytic anaemia in overt lupus erythematosus has responded favourably to splenectomy [74].

Looking at the impressive list of different haemolytic disorders, it is obvious that no effective therapy is available for most of them. I should like therefore to restrict my comment to a few of these states. The haemolytic anaemia associated with virus infections, for example primary atypical pneumonia and mononucleosis, may be severe but it is always a self-limited disease. Transfusions may be used to tide the patient over

the dangerous period. Prednisone has been used with good results, and without an apparent aggravation of the virus infection.

The most rewarding results f splenectomy are obtained in hereditary spherocytosis. The results are so consistently favourable that therapeutic failure indicates either that the initial diagnosis was wrong or that accessory spleens are present. It is difficult to make a specific recommendation concerning the best time to perform splenectomy. Jaundice the presence of gallstones, and anaemia requiring regular transfusions may be considered absolute indications at any age. Unless the patient illness is completely compensated and the patient quit symptom-free it would seem wise to remove the spleen before the age of 7 8 years, after hich the incidence of gallstones has been reported to increase rapidly. In congenital non-spherocytic haemolytic anaemia cort costeroid are without effect. Some cases will derive benefit from splenectomy

In thalassaemia major splenectomy has been reported to lessen the transfusion requirement in selected cases. Patients with sickle-cell anaemia as a rule seem to derive little benefit from splenectomy

Auto-immune haemolytic anaemia poses the most difficult problems w th regard to therapy The im of treatment in this stat is to keep the patient live until a temporary or lasting remission occurs. It has been shown in large series collected by for example Dacie and associates in London [11], and Dausset group in Paris [13] that the cure rate is about 50 %.

Unfortunately it is impossible t predict which patients are going to recover The therapeutic pproach consists of initial treatment with high doses of prednisone or ACTH If the patient fails t respond, or if there are complications or contraindications t this therapy splenectomy should be considered. As mentioned before investigation f the accumulation of radioactive chromium in the spleen and liver is desirable but is not f crucial importance for determinating whether splenectomy should be performed. If splenectomy is unsuccessful, prednisone or ACTH may be tried again. If they fail once more which is probable the patient is in a sad situation. There remain only blood transfusions, which t this stage are frequently complicated by reactions. These may be diminished by using washed red-cell con centrat instead of whole blood. Although the prognosis for these patients is poor it is not without hope There re al ys the chances that the disease will die before the patient.

References

1 ABRAHAMOV A and D AM L K. Erythrocyte glycolysis in erythroblastotic newborns. Amer J Dis Child 99 383, 1960
2 ADNER, P L and FOCK S Unexpected blood group mosaics but revealed by 51C labelled red cell Scand J Clin Lab Invest 9 265, 193
3 AGER, J A M LEHMA H and VELLA F Haemoglobin Norfolk new haemoglobin found in an English famil Brit Med J 839 1958
4 ALLISO A C Acut haemoly ic anaemia ith distortion and fragme ta ion of ery hroc es in children Brit J Haemat 1 1 195
5 ALTM K J TABECHIA H and Yo L F Some aspect of he met bolism of red blood cells from patients ith hemolytic anemias J V Amer Med 1 142 195
6 A TM K J Some enzyme Some aspect of the human erythrocyte. Amer J Med 27 936 1959
7 AUDITORE, J V HARTMANN R C and C F F Potassium transport in the acetylcholinesterase-deficient erythrocytes of par xysmal nocturnal hemoglobinuria (PNH). J Cl I vest 38 70, 1959
8 BERNOWSKY I and J CONSEN L Hereditär benign erythroretikulose Sc and L Akertid 4 1183 1959
9 BEUTLER, E The hemolytic effect of primaquine and related compound review Blood 14 103, 1959
1 BIRTFIELD A J and D I Atypical congenital hemolytic anemia. Arch Dis Child 1 83, 1957
11 CRITTENDEN O and D J V Result of splenectomy in immune haemolytic anaemia, Brit J Haemat, 1 237 1958,
12 DAUSSET J COLIN R D AN J TAPALO OT

A., MATHÉ, G HARIS, R. and DESMONBLLE, M. Purpura thrombotique thrombocytopénique (maladie de Moschcowitz) chez une enfant de dix mois. *Presse Méd* 65 2144 1957

13. COCK, TH C.: Acute hemolytic anemia in neonatal period. *Amer J Dis Child*, 94 77 1957

14. COHEN J A. and WARRINGA M. G P J.: Fat of P[32] labelled diisopropylfluorophosphat in human body and its use as a labelling agent in study of turnover of blood plasma and red cells. *J Clin Invest* 33, 459 1954

15. COOMBS, R. R. A. and ROBERTS, G FULTON Antiglobulin reaction. *Brit Med Bull* 15 113 1959

16. CROSBY W H. and AKEROYD J H.: The limit of hemoglobin synthesis in hereditary hemolytic anemia. *Amer J Med*, 13 273, 1952.

17. DACIE, J C Differences in the behaviour of sensitized red cells to agglutination by antiglobulin sera. *Lancet* 954, 1951

18. DACIE, J V *The Haemolytic Anaemias Congenital and Acquired* Churchill Ltd., London, 1954

19. — *The Haemolytic Anaemias Part I The Congenital Anaemias* 2nd edition, Churchill Ltd London, 1960

20. — *Acquired haemolytic anaemias. Brit Med Bull* 15 67 1959

21. DAMESHEK, W Hypersplenism *Bull N Y Acad Med* 31 113, 1955

22. DAUSSET J and COLOMBANI J The serology and the prognosis of 128 cases of autoimmune hemolytic anemia, *Blood*, 14 1280 1959

23. DESFORGES, J F KALAW E. and GILCHRIST P Inhibition of glucose 6-phosphate dehydrogenase by hemolysis inducing drugs *J Lab Clin Med*, 5 75 1960

24. DUNHAM E T and G[illegible] I M. Adenosinetriphosphatase activity and ion movements *J Physiol* 1 5 61P 1960

25. DUNSFORD I and GRANT J *The Antiglobulin (Coombs) Test in Laboratory Practice* Oliver and Boyd Edinburgh London, 1959

26. ENGSTEDT L. Endogenous formation of carbon monoxide in hemolytic disease *Acta Med Scand* 154 Suppl 33 185

27. ERLANDSON M F and HILGARTNER, M Hemolytic disease in the neonatal period and early infancy *J Pediat* 4 556 1959

28. EVANS, R. S. and WEISER, R S The serology of autoimmune hemolytic disease; observations on forty-one patients *Arch Int Med*, 100 371 1957

29. FOCONI, S and SJÖLIN S Survival of Cr[51] labelled red cell from new-born infant *Acta Pædiat*, 48 S ppl 117 18 1959

30. GARBY L., SJÖLIN S. and VAHLQUIST B Chronic refractory hypochromic anaemia with disturbed haem-metabolism. *Brit J Haemat* 3 55, 1957

31. GIBLETT E. R., COLEMAN D H PIRZIO-BIROLI, G DONOHUE, D M., MOTULSKY A G and FINCH, C. A.: Erythrokinetics Quantitative measurements of red cell production and destruction in normal subjects and patients with anemia. *Blood*, 11 291 1956.

32. GILBERTSEN S HAWKINSON V and WATSON C. J.: Studies of the dipyrrylmethene ("Fuscin") pigments. II. The contrasting ratios and significance of the fecal urobilinogen and mesobilifuscin in certain anemias. *J Clin Invest* 38 1175, 1959.

33. GILBERTSEN S LOWRY P T HAWKINSON V and WATSON C. J.: Studies of the dipyrrylmethene ("Fuscin") pigments. I. The anabolic significance of the fecal mesobilifuscin. *J Clin Invest* 38 1166, 1959.

34. GOFSTEIN R. and GELLIS, S. S.: Splenectomy in infancy and childhood. *Amer J Dis Child* 91 566, 1956.

35. GREEN N and GOLDENBURG, H.: Acute hemolytic anemia and hemoglobinuria complicating infectious mononucleosis. *Arch Int Med* 105 108, 1960

36. GREENWALT T J and AYERS, V E.: Phosphate partition in the erythrocytes of normal newborn infants and infants with erythroblastosis fetalis II. Quantitative paper chromatography *Blood*, 15 [illegible], 1960

37. GROSS, R. T HURWITZ, R. E. and MARKS, P A. An hereditary enzymatic defect in erythrocyte metabolism: glucose-6-phosphate dehydrogenase deficiency *J Clin Invest* 37 1176 1958.

38. GRUBB R.: Agglutination of erythrocytes coated with incomplete anti Rh by certain rheumatoid arthritic sera and some other sera. The existence of human serum groups. *Acta Path Microbiol Scand* 39 195, 1956.

39. GRUCHY DE, G C *Clinical Haematology in Medical Practice* Blackwell, Oxford, 1958

40. HANSEN H A J ONSTRUCHO, O R. and J HANSSON B. G Studies on an abnormal hemoglobin causing hereditary congenital cyanosis. *Acta Pædiat* 49 503, 1960.

41. HEDENBERG, F MÜLLER EBERHARD, U SJÖLIN S and WRANNE, L. Haemoglobin H and inclusion-body anaemia in Swedish family *Acta Pædiat* 47 652, 1958

42. HELLANDER, L. and B TRECKELT E.: Beitrag zur Spezifität antierythrocytärer Antikörper Proc 6th Congress Europ Soc Haemat, p. 680 Copenhagen 1957

43. HEWELL, O R., MALCOLM, J M and PIKE, R The F II-agglutinating factor in serums of patients with non-rheumatic diseases. *Amer J Med* 29 64, 1960

44. Hughes-Jones N C Mollison P L and V all, N: Removal of incompatible red cells by the spleen. Brit J Haemat 3 1 5, 1957
45. Högman C., Killander, J and Sjölin S A case of idiopathic autoimmune haemolytic anaemia due t anti-e Acta Paediat 49 ... 1960.
46. Ingram, V M: Chemistry of the abnormal human haemoglobins. Brit Med Bull 15 27 1959.
47. Ivemark, B I: Implications of agenesis of the spleen on the pathogenesis conotruncus anomalies in childhood Acta Paediat 44 Suppl 104 1955
48. Jandl, J H Jones, R. and Castle W B The destruction of red cells by bodies in man. I Observations on the sequestration and lysis of red cells altered by immune mechanisms. J Cl Invest 36 1428, 1957
49. Jandl, J H. and T mollison A S The destruction of red cell by ant bodies in man II. Pyrogenic leucocytic and dermal re sponses to immune hemol sis J Cl Invest 37 1202, 1958.
50. Jandl, J H. and Kaplan M E The de struction of red cells by antibodies in man. III. Quantitative factors influencing the patterns of hemolysis in vivo J Clin I vest 39 1145, 1960
51. Jenkins K. O Elution of incomplet ant bodies from red cells A comparison of dif ferent methods. Vox Sang 4 230, 1959
52. Kin H. and Schumacher, H B Jr.: Splenic studies I. Susceptibilit to infection after splenectomy performed in infancy Ann Surg 136 239 1952.
53. Kirvit W Smith, R T Mar n J F Rea R. and Good R A Congenital nonspherocytic hemolytic anaemia T non familiar cases with red cell survival studies J Pediat 49 245, 1956
54. Lakey, B and Ma Milla A Incidence of infection in children after splenectom Pediatrics 22 523, 1958.
55. Lakey, B Wake, E Gunson H and Bain H. W Idiopathic acquired hemolytic anaemia in two young infants. Amer J Dis Child, 100 524 1960
56. Lelo M Alla ulf D and Dormon J La forme ictérique néonatale de la ma ladie de Minkowski-Chauffard Arch Franc Pédiat 16 877 1959
57. Löhr, G W and Waller, H D Hämolytische Erythrocytopathie durch F hlen von Glukose-6 Phosphatdeh drogenase in roten Blutzellen al dominant vererbliche Krankheit Klin Wschr 36 865, 1959
58. McKinnon W M P Boley S J and Manfel, J Infection in children following splenectomy for traumatic rupture Amer J Dis Child. 97 710 1959
59. Mills J N The life span of the erythrocyt J Physiol 105 16 (P) 1946
60. Mollison P L: Blood Transfusion i Cli ical Medicine 2nd edition, Blackwell Scientific Publications, Oxford, 1956.
61. — Red cell survival in haemolytic syn dromes. Brit Med Bull 15 59 1959.
62. — Blood-group antibodies and red-cell de struction. Brit Med J 1 1036, 1123, 1956
63. Murphy J R.: Erythrocyt metabolism. II Glucose met bolism and pathw ys. J Lab Clin Med 55 286, 1960
64. Nosslin B F and Nyman M: H ptoglobin determination in diagnosis of haemolytic diseases. Lancet 1, 1000, 1958.
65. P uling, L., Itano, H. A., Singer, S. J and Wells, I. C.: Sickle cell anemia, molecular disease Science 110 543, 1949
66. P uling, L., I ano H. A., Wells, I. C Schroeder, W A., Kay L. M Singer, S. J and Corey R. B Sickle cell anemia hemoglobin Science 111 459, 1950.
67. Post M. E Jones, J A. and Butler J A.: Exchange transfusion in neonatal crisis of congenital hemolytic anemia. Amer J Dis Child 99 231 1960
68. Post R. L., Merritt C R., Kinsolvin C R and Albright C D Membrane adenosine triphosphatase as participant in the active transport of sodium and potassium in the human erythrocyt J Biol Chem 235 1796 1960
69. Prankerd, T A. J Inborn errors of metabolism in red cells of congenital hemolytic anemias. Amer J Med 22 724, 1957
70. Prankerd T A.: Red-cell structure and met bolism in haemolytic anaemia. Brit Med Bull 15 54 1959
71. Rapoport S. M.: Glutathione stability and pyrophosphatase activity in reticulocytes Direct evidence for the importance of glutathione for the enzyme status in intact cells. Nature 186 967 1960
72. Robinson T W and Sturgess P Post splenectomy infection in infant and chil dren Pediatrics, 25 941 1960
73. Rosenfield R. E Haber, G N and Gilbert H. S Complement and the antiglobulin test Vox Sang, 4 182, 1960.
74. Sackler, H E. and Levin W C The role of splenectomy in the management of acquired autoimmune hemolytic anemia complicating systemic lupus erythematosus Amer J Med, 24 547 1958.
75. Schalck D and Rapoport S M.: Glutathione and Triosephosphatdehydrogenase Aktivität in roten Blutzellen on Kaninchen. Klin Wschr 38 757 1960
76. Selwyn J G and D cie, J V Autohemolysis and other changes resulting from the incubation in vitro of red c ... tients with congenital ... Blood 9 414, 1954

77 Shahidi, N T and Diamond L. K.: Enzyme activity in erythrocytes in congenital nonspherocytic hemolytic anemia. *Pediatrics*, 4 245 1950.
78 Shapiro, Ch. M., Josephson A. M., Rosenfield, R. and Kauffman A.: Hereditary spherocytosis in neonatal period: Diagnosis, incidence and treatment *J Pediat* 50 305, 1957
79 *Siedel, W Stich, W and Eisenreich, F.:* Pronemobilifuscin (Mesobilileukan), ein neues physiologisches Abbauprodukt des Blutfarbstoffes. *Naturwissenschaften*, 35 316 1948.
80 Sjölin S The resistance of red cells in vitro. A study of the osmotic properties, the mechanical resistance and the storage behaviour of red cells of fetuses, children and adults. *Acta Paediat 43 S uppl. 98* 1954
81 Sjölin S Anstee, P L. and Fogarty, S.: ^{51}C -labelled red cells as means of detecting small quantities of ant bodies Proc th Congr Europ. Soc Haemat., London 1959 part II 1129 1960
82. Sjölin S.: Studies on osteopetrosis. II Investigations concerning the nature of the anaemia. *Acta Paediat* 48 529 1959
83. Sjöstrand T *Endogenous formation of* carbon monoxide in man. *Nature 164* 580, 1949
84. Smith, C. H., Erlandson M Schulman I. and Stern G Hazard of severe infections in splenectomized infants and children. *Amer J Med*, 22 390 1957
85 Stewart J W and Mollison P L. Rapid destruction of pparently compat ble red cells. *Brit Med J* 1274 1959
86. Storti, E., Vaccari, F and Baldini, E.: Changes in red cells metabolism in presence of incomplet ant bodies. *Experientia 12* 108, 1956
87 Todd R. M Lamb and O Donohoe, N V Acut acquired hemolytic anemia associated with herpes simplex infection *Arch Dis Child*, 35 5.4, 1958.
88. Trout S B Swisher, S. N and Young, L. E.: The anemia of leukemia. *Amer J Med*, 28 751 1960.
89 Tuffy Ph., Brown A. K. and Zuelzer, W W.: Infantile pyknocytosis. *Amer J Dis Child*, 98 227 1959
90. Wallenius, G and Höglund, N J Personal communication.
91 Weiner, W Battey D A., Cleghorn, T E., Marson F G W and Meynell, M. J Serological findings in case of haemolytic anaemia with some general observations on the pathogenesis of this syndrome *Brit Med J* 2 125, 1953.
92. Wilk, S. A. and Strumpf Ph Thrombotic thrombocytopenic purpura: Review of subject with report of three cases in children. *Pediatrics* 17 882, 1956.
93 Wintrobe, M. M.: *Clinical Hematology* Fourth edition. Lea & Febiger Philadelphia, 1956.
94. Wolfe, J W Brin M. and Davidson C. S.: The effect of thiamine deficiency on human erythrocyt met bolism. *J Clin I vest* 37 1476, 1958
95. Zetterström, R. and Strindberg, B.: Sporadic congenital spherocytosis associated with congenital hypoplastic thrombocytopenia and malformations. *Acta Paediat* 4 14, 1955.
96 Zinkham, W H. and Childs, B.: Defect of glutathione metabolism in erythrocytes from patients with naphthalene-induced hemolytic anemia. *Pediatrics*, 22 461 1958.
97 Zinkham, W H An in-vitro bnormality of glutathione metabolism in erythrocytes from normal newborns mechanism and clinical significance *Pediatrics* 23 18, 1959.
98 Zinkham W H. and Lenhard R. E., J Met bolic abnormalities of erythrocytes from patients with congenital nonspherocytic hemolytic anemia. *J Pediat* 45 310, 1959

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SUMMARY OF SUPPLEMENTS

Pneumoencephalography in Atrophic Brain Lesions in Infancy and Childhood

by J C. MELCHIOR

(Supplement 127)

The purpose of the present study is to examine the prognostic value of pneumoencephalography (P E G) in a number of childhood disorders causing either real or apparent dilatation of the ventricula system The value of certain measuring methods is examined and the reliability of one such method is ascertained

A survey of the literature on P E G is made and this is divided into four parts The first reviews its development with special reference to experimental examinations results and complication in adults In the second part reports concerning paediatric examinations are mentioned and in the third the development of P E G in Denmark is briefly reviewed. The pi ture revealed is that of tendency for in terest to fluctuate over the 40 years At the beginning excessive enth siasm wa resisted strongly In the next ten years there wa little critical valuation of the examination but this wa followed by creasing interest in factors influencing complications In the last ten years the use of air insufflation has increased, in other field as well in tumour diagnosis and there has been more critical evaluation of the results obt ined In th fourth section the different measurements which are used to evaluate the degree of dilatation of the ventricular system are discussed. These method are divided into planimetric and linear measurements and are given as exact or relative values. The P E.G measurements in suitable cases can be compared with autopsy material. The most frequently used figures are Evans ratio and Schiersmann s index and these are discussed in detail and it is emphasised that the use of relative values are of particular importance in childhood where the exact figures vary very much

The terminology and abbreviation used n the text re discussed. The present material is made up of *71 children (171 boys and 100 girls) To be included in the material the following criteria had to be full filled.

(i) All patients were under fiteen years of age at the time of examination u the Neurosurgical Department

(i) Tumours and cut infection of the C.N.S were excluded

(iii) P.E.G wa performed either n the Neurosurgical Department r im mediat ly before admission

logically normal joints. In the remaining 5 cases the continued development of the joint will probably be normal in a further 2 The percentage of normal hips will then be 92 3 %. One case had dysplastic changes to a somewhat greater degree and two showed results of capital necrosis. Their future development is at present uncertain.

The cases thus diagnosed are regarded as representing a stage of preluxation in the newborn. Left untreated, a number of these cases may heal spontaneously and others may give rise to subluxation or luxation during the first to second year of life in milder cases not until later in childhood, or adult years in the form of arthrosis deformans (osteoarthritis)

A critical study is made of the roentgen examination of the newborn, and in particular its sources of error This examination revealed nothing in cases of preluxation in addition to that which could be established clinically Roentgen examination of these cases before the commencement of treatm nt is therefore considered unnecessary

An examination of a normal material of newborn was made in respect to the possible degree of abduction in the hip joints and the symmetry of the skin fold on the medial side of the thigh All had an abduction of at least 70° Only 30 6 had symmetrical thigh folds. 7 6 had no folds at all these being mainly children with a low birth weight Asymmetry of the skin folds thus does not appear to be a symptom of value in the diagnosis

Most cases of preluxation have normal abduction, and many have increased flaccidity of the joint

At the suggestion of the author the examination of the hip joints was introduced in January 1953 into the routine examination of all newborn children at the majority of obstetric clinics having pediatric consultants. Up to and including 1960 approximately 415,600 newborn children were examined, constituting 49.5 % of all children born alive in Sweden during this period. During 1959 and 1960 approximately 67 % were examined. This material was analyzed. Altogether 804 cases of preluxation were diagnosed (=2.2‰) During the later years this frequency was about 3‰. In those clinics which also used the subluxation provocation method in the examination during the later years, the frequency was about 5–6 ‰. No obvious geographical differences in the frequency within Sweden was found in this material.

In order to study how the diagnoses made at the obstetric clinics have affected the frequency of luxation during later childhood, and thereby the usefulness of this diagnosis, all cases of luxation of the hip joint in children treated at the orthopedic clinics in Sweden during the years 1948–1960 1486 cases altogether were recorded. (In Sweden practically all cases of luxation are treated at orthopedic clinics) The ages of the children at the time the diagnosis was made and the reasons leading to the examination of the hip joints, are given.

During the later years an increasingly large number of newborn children were transferred to the orthopedic clinics, but in spite of this there was no marked increase in the number of cases treated annually at these clinics.

Approximately 62 % of the preluxation cases were treated at the pediatric clinics the majority as out patients.

Of the cases of luxation admitted to orthopedic clinics during the years 1948–1950 none had been diagnosed when new born, and 85 % were not diagnosed until after the age of 1 year. Ninety-seven per cent underwent the customary prolonged treatment with plaster.

Of the 229 cases treated during the years 1959–1960 69 % were diagnosed at obstetric clinics, and only 23 % after the age of 1 year. It was possible for 58 % to be treated as out-patients with a simple method of fixation in an abduction position for a period, in the majority of cases, of only a few months. Thirty nine per cent were treated with plaster.

The material from the orthopedic clinics was compared with that from the obstetric clinics, and in the cases of luxation from the orthopedic clinics inquiry was made as to the obstetric clinic at which the child was born. It was thereby found that during the years 1953–1960 114 children were admitted, who were not diagnosed as preluxations when newborn, but who were born at those clinics where the hip joints were examined as routine. Ten cases had been transferred to a pediatric clinic because of prematurity, asphyxia or malformations, and had possibly therefore had no hip joint examination immediately. It was not possible to verify with certainty whether all of the other cases had been examined when newborn.

An obvious tendency was observed in the number of cases of later manifested luxation which was not discovered in the neo-natal period, to decrease with the years, when the interest and experience of the examiners had extended.

The material from those clinics which have shown especial interest and have considerable experience reveals that at least 90 % of those dislocations which may be expected to occur during later childhood years can be diagnosed as preluxations during the first week of life. In this way they are also available for immediate treatment with simple fixation in the abduction position, and in probably at least 90 % the subsequent development of the joint is completely normal.